

Biochemistry

Debajyoti Das

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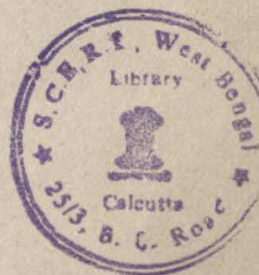
Human Physiology

BIOCHEMISTRY

DEBAJYOTI DAS

Presidency College, Calcutta

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In memory of
PARIMAL BIKAS SEN
my teacher

SOME UNITS USED

Reciprocal units are frequently given in this book according to the SI convention, replacing both the stroke and *per* between two units by a negative index ; e.g., mg dl^{-1} , $\text{kg m}^{-1} \text{ sec}^{-1}$, $\text{kcal kg}^{-1} \text{ hr}^{-1}$.

micrometre (μm) = 10^{-3} mm

nanometre (nm) = 10^{-6} mm

microgram (μg) = 10^{-3} mg

nanogram (ng) = 10^{-6} mg

picogram (pg) = 10^{-9} mg

millipoise (mP) = 10^{-4} $\text{kg m}^{-1} \text{ sec}^{-1}$

megajoule (MJ) = 10^6 $\text{m}^2 \text{ kg sec}^{-2}$

svedberg (S) = 10^{-13} cm sec^{-1}

litre (l) = 1 dm^3

millilitre (ml) = 10^{-3} l

decilitre (dl) = 10^{-2} ml

microlitre (μl) = 10^{-3} ml

kilocalorie (kcal) = 10^3 cal

PREFACE TO THE SIXTH EDITION

This book is intended for students of Medical and Biological Sciences, Biochemistry and related curricula. To serve that purpose better, all chapters of the present edition have been very extensively revised, reoriented and rewritten in the light of recent advances in Biochemistry. Many sections on hitherto undiscussed topics have been incorporated in most of the chapters. Clinical orientations have been retained. Methods for separating proteins, nucleic acids, amino acids and lipids have been described more exhaustively. Regulations and inborn errors of all important metabolic pathways have been discussed. Many topics of immunology such as immunoglobulins, complements, anaphylactic agents, lymphokines and biochemical changes in phagocytosis have been dealt with. The chapter on Cell Biology has been dispensed with, but important topics of that chapter such as membrane, electron transport chains of membranes, cell junctions, chromatin and glycosylation of membrane proteins have been described elaborately in the relevant sections of other chapters. Except a limited few, all diagrams have been replaced by new ones conforming to the modern concepts. The number of diagrams has also been considerably increased.

I am indebted to Professor H. Y. Mohan Ram, Professor S. Mahadevan, Professor Jagat Jiban Ghosh, Professor Manju Mukherjee, Professor Malaya Gupta, Professor Madhusudan Ghosal and Dr. Asoke Gopal Datta for their kind appreciation of my work. Dr. Gourisankar Sa and my daughter Dr. Tanya Das helped me by criticizing some portions of the manuscript. I am grateful to Mr. Bimal Kumar Dhur for publishing this book. I have always received inspiration and encouragement from Mr. Rajendra Nath Dhur, my wife Arati and my son Sayan. My father Byomkesh Das had been taking keen interest in all my work; I feel very sad that he passed away before the publication of this edition.

Debajyoti Das

Presidency College
Calcutta
12 March 1990

PREFACE TO THE FIRST EDITION

For several years, Professor Parimal Bikas Sen and Shri Rajendra Nath Dhur were urging me to write a book on Biochemistry. I am indebted to them for the confidence they induced in me. I feel sad that Professor Sen passed away before I could start writing this book.

This book endeavours to lead the reader from a rather elementary stage to a somewhat advanced level. It aims to serve relatively advanced students of the general courses in Zoology, Botany, Physiology and Biochemistry as also of the medical and veterinary sciences. Attempts have been made here to give the reader an insight into the biochemical aspects of life processes including the rate-limiting reactions and regulations of metabolic pathways, inborn errors of metabolism, roles of isozymes of the same enzyme in guiding the metabolism through diverse pathways, genetic code and the translation of the coded messages into peptide chains, metabolic functions and modes of actions of vitamins and hormones, prostaglandins and biological oxidations. Some important clinical tests like the immunological test for pregnancy, carbohydrate tolerance tests and liver function tests have been mentioned. In the short chapter on human nutrition, important Indian foods, protein-calorie malnutrition, basic principles of dietetics and the procedure for calculating individual dietary needs have been discussed. Specific diets to suit Indians have also been formulated. Digestion in ruminants and birds, production of uric acid as the endproduct of proteins in uricotelic animals, water-conservation in arid-zone animals, crop-milk, differences between procaryotes and eucaryotes in many metabolic pathways, gluconeogenesis and liponeogenesis in ruminants and differences in hormone secretions in different animals constitute some of the comparative aspects that have been discussed with the intention of helping the students of Zoology, Botany and Veterinary science.

I am particularly grateful to Dr. Asoke Gopal Datta (Indian Institute of Experimental Medicine) for his valuable suggestions and constructive criticisms. I am also indebted to Dr. Arup Kumar Sinha (Presidency College), Dr. Debi Chakravarty (Bethune College), Dr. Kanak Sankar Ray (Nirnayan Pathological Laboratory), Dr. Parimal Ray (Moulana Azad College), Dr. Pijush Kanti Das (Hooghly Mohsin College), Dr. Robin Prasad (Hooghly Mohsin College) and Dr. Simananda Adhikari (Moulana Azad College) for the constant encouragement received from them.

I am grateful to Shri Bimal Kumar Dhur and Shri Lakshman Kumar Ghosh of Academic Publishers for their sincere efforts for the publication of this book. Dr. Subhendusekhar Mukhopadhyay (Sahitya Akademi) has all along inspired me by taking a keen interest in my work. My daughter Tanya has helped me in the toilsome work of proof-reading.

Debajyoti Das

12 February 1978

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ABBREVIATIONS FREQUENTLY USED

ACP acyl-carrier protein. ACTH corticotropin or adrenocorticotropin: hormone. ACU adult consumption unit. Acyl-CoA fatty acid-coenzyme A. ADP adenosine diphosphate. ALA delta-aminolevulinate. Ala alanine. AMP adenosine monophosphate or adenylate. Arg arginine. Asn asparagine. Asp aspartate. ATP adenosine triphosphate. AUG adenylate-uridylylate-guanylate. BMR basal metabolic rate. cAMP cyclic AMP. CCA cytidylate-cytidylate-adenylate. CCK cholecystokinin. CDP cytidine diphosphate. Cer ceramide. CMP cytidine monophosphate or cytidylate. CRH corticotropin releasing hormone. CTP cytidine triphosphate. Cy cytochrome. Cys cysteine. dAMP deoxyadenylate. dCDP deoxycytidine diphosphate. dGTP deoxyguanosine triphosphate. dTMP deoxythymidylate. dUMP deoxyuridylylate. DHCC dihydroxycholecalciferol. DIT diiodotyrosine. DOPA dihydroxyphenylalanine. ECF extracellular fluid. EFA essential fatty acid. Eq equivalent. ER endoplasmic reticulum. FAD flavin adenine dinucleotide. FFA free fatty acid. FMN flavin mononucleotide. FSH follicle stimulating hormone. GAC guanylate-adenylate-cytidylate. Gal galactose. GalNAc N-acetylgalactosamine. GDP guanosine diphosphate. GFR glomerular filtration rate. GH growth hormone. GHRH GH-releasing hormone. GIP gastric inhibitory peptide. Gla gamma-carboxyglutamate. Glc glucose. GlcNAc N-acetylglucosamine. Gln glutamine. Glu glutamate. Gly glycine. GMP guanosine monophosphate or guanylate. GnRH gonadotropin releasing hormone. GSH glutathione. GTP guanosine triphosphate. Hb hemoglobin. HCC tetrahydrofolate. His histidine. HMG β -hydroxy- β -methylglutarate. HMP hexose monophosphate. HRE hormone responsive element. IDDM insulin-dependent diabetes mellitus. IDL intermediate-density lipoprotein. IgG immunoglobulin G. Ile isoleucine. IMP inosine monophosphate or inosinic acid. IU international unit. LDH lactate dehydrogenase. LDL low-density lipoprotein. Leu leucine. LH luteinizing hormone. LTA leukotriene A. Lys lysine. Man mannose. Met methionine. MIT monoiodotyrosine. mRNA messenger RNA. MSH melanocyte stimulating hormone. MW molecular weight. NAD⁺ nicotinamide adenine dinucleotide. NADP⁺ NAD⁺-phosphate. NANA or NeuAc N-acetylneuraminic acid. NHI non-heme iron. NIDDM non-insulin-dependent diabetes mellitus. NMN nicotinamide mononucleotide. NPN non-protein N. PAH para-aminohippurate. PBI protein-bound iodine. PDH pyruvate dehydrogenase. PEP phosphoenolpyruvate. PEPCK phosphoenolpyruvate carboxykinase. PFK phosphofructokinase. PGE prostaglandin E. Phe phenylalanine. Pi inorganic phosphate. PLP pyridoxal phosphate. PPi inorganic pyrophosphate. PP-ribose-P/PRPP 5-phosphoribosyl-1-pyrophosphate. PRL prolactin. Pro proline. PTH parathyroid hormone. RQ respiratory quotient. rRNA ribosomal RNA. SDA specific dynamic action. Ser Serine. T₃ triiodothyronine. T₄ thyroxine. TCA tricarboxylic acid. Thr threonine. Tm transport maximum. TMP thymidylate. TpC troponin C. TPP thiamin pyrophosphate. TRH thyrotropin releasing hormone. tRNA transfer RNA. Trp tryptophan. TSH thyroid stimulating hormone or thyrotropin. TXA thromboxane A. Tyr tyrosine. UAA uridylylate-adenylate-adenylate. UAG uridylylate-adenylate-guanylate. UDP uridine diphosphate. UGA uridylylate-guanylate-adenylate. UMP uridine monophosphate or uridylylate. UTP uridine triphosphate. Val valine. VIP vasoactive intestinal peptide. VLDL very low-density lipoprotein.

1. CHEMISTRY OF CARBOHYDRATES

Some carbohydrates form structural elements of the living body ; e.g., cellulose, hemicellulose, pectin, chondroitin sulfate, hyaluronic acid and chitin. Some other carbohydrates are meant for energy production ; e.g., starch, dextrin, glycogen, trehalose and glucose. Carbohydrates also form complexes with non-carbohydrates including proteins, lipids, purines and pyrimidines.

1.1 DEFINITION

Generally, carbohydrates or their hydrolytic products are either polyhydroxy aldehydes or polyhydroxyketones. So, each carbohydrate molecule contains many hydroxyl groups and one or more aldehyde or ketone groups. The aldehyde or ketone group may remain in either free or bound form in the molecule.

Carbohydrates generally have a molecular formula of $C_nH_{2n}O_n$ or $[C_nH_{2n-2}O_{n-1}]_x$. But all carbohydrates do not have such a formula ; e.g., fucose ($C_6H_{12}O_5$) and rhamnose ($C_6H_{12}O_5$). Nor can all substances with such a molecular formula be called carbohydrates ; e.g., formaldehyde (CH_2O), lactic acid ($C_3H_6O_3$) and acetic acid ($C_2H_4O_2$). Some carbohydrates contain nitrogen as amino group ; e.g., glucosamine and galactosamine ($C_6H_{11}O_5.NH_2$). Some also contain sulfur as sulfate group ; e.g., glucosamine 6-O-sulfate [$C_6H_{10}O_6(SO_3^-)NH_2$].

1.2 CLASSIFICATION

Carbohydrates are divided into two main classes.

1. Monosaccharides or simple sugars : These are the simplest of carbohydrates and

cannot be hydrolyzed into smaller carbohydrate molecules. Most of the monosaccharides have the general formula $C_nH_{2n}O_n$. Monosaccharides of biological relevance bear a carbon skeleton of 3-7 carbons.

Monosaccharides are usually classified according to the number of carbons in the molecule : (a) *trioses* ($C_3H_6O_3$), e.g., glyceraldehyde and dihydroxyacetone ; (b) *tetroses* ($C_4H_8O_4$), e.g., erythrose and erythrulose ; (c) *pentoses* ($C_5H_{10}O_5$), e.g., ribose and xylulose ; (d) *hexoses* ($C_6H_{12}O_6$), e.g., glucose, galactose, fructose and mannose ; (e) *heptoses* ($C_7H_{14}O_7$), e.g., sedoheptulose.

2. Compound carbohydrates : They are formed by the linking of two or more monosaccharide molecules with glycoside bonds. They can be hydrolyzed into more than one monosaccharide molecule and are classified as follows :

(a) *Oligosaccharides* : They are composed of 2-6 monosaccharide molecules united by glycoside bonds. They are divided into classes such as (i) *disaccharides*, each formed by the union of 2 monosaccharide molecules, e.g., sucrose, maltose, lactose and trehalose, (ii) *trisaccharides*, each composed of 3 molecules of monosaccharides, e.g., raffinose and maltotriose, and so on.

Sugars are monosaccharides and oligosaccharides which form sweet, water-soluble crystals, insoluble in most nonpolar solvents.

(b) *Polysaccharides or glycans* : They are macromolecular carbohydrates. Each polysaccharide molecule is composed of many molecules of monosaccharides (e.g., glucose, fructose or galactose) and modified or derived monosaccharides (e.g., galacturonic acid or

N-acetylglucosamine). Polysaccharide is formed by the linking of many sugar molecules by glycosidic linkages. Polysaccharides have two subclasses: (i) *homopolysaccharides* or *homoglycans*, e.g., starch, glycogen and inulin, are each composed of many molecules of the same monosaccharide; (ii) *heteropolysaccharides* or *heteroglycans*, e.g., hyaluronic acid and heparin, are each composed of more than one type of monosaccharide. Homoglycans composed of hexose molecules are called *hexosans*. According to the type of hexose molecule, they may also be called *glucosans* (e.g., starch and glycogen, made of glucose), *fructosans* (e.g., inulin made of fructose), etc.

1.3 ISOMERISM OF MONOSACCHARIDES

Different types of isomerism are found in monosaccharides. Compounds with the same molecular formula but different structural formulae are isomers of each other.

1. **Aldose-ketose isomerism:** Monosaccharides possess either an aldehyde or a ketone group in the molecule. *Aldoses* are monosaccharides having an aldehyde group ($\text{H}-\text{C}=\text{O}$) at one end (C_1) of the molecule; e.g., glucose,

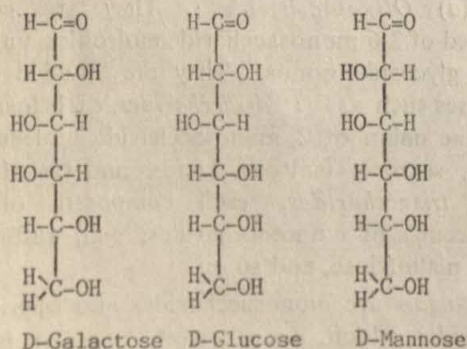


Fig. 1.1. Some aldohexoses.

galactose (Fig. 1.1). *Ketoses* have a ketone group ($\text{C}=\text{O}$) at a position other than C_1 ; e.g., fructose (Fig. 1.2). Aldose-ketose isomerism is the existence of an aldose and a

ketose with the same molecular formula. Glucose is an *aldohexose* (aldehyde-bearing hexose) and fructose is a *ketohexose* (ketone-bearing hexose); so, they are aldose-ketose isomers of one another. Similarly, ribose (an *aldopentose*) and ribulose (a *ketopentose*) are aldose-ketose isomers.

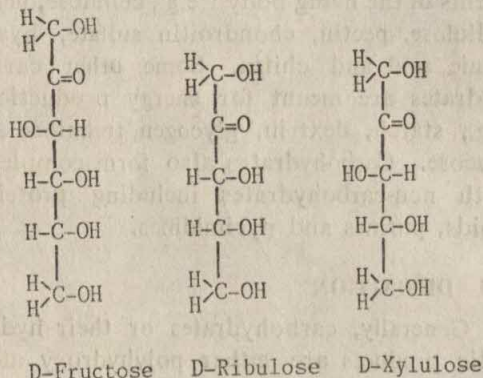


Fig. 1.2. Some ketoses.

2. **Stereoisomerism:** An asymmetric carbon carries four different groups or atoms on its four valencies (Fig. 1.3). Stereoisomerism is the existence of the same compound

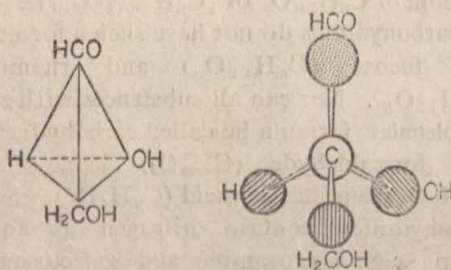


Fig. 1.3. Models of D-glyceraldehyde showing groups on its asymmetric carbon.

in more than one form due to different spatial arrangements of the groups or atoms attached to its asymmetric carbons (Fig. 1.4). Each sugar with a specific spatial arrangement of its groups may have its exact mirror image in another isomer (e.g., L-arabinose and

D-arabinose in Fig. 1.4). These mirror images are called *D* and *L* forms of that sugar. The *D* isomer has the H and OH groups of its penultimate carbon atom (next to the terminal CH_2OH -carbon) oriented just like those around the asymmetric middle carbon of

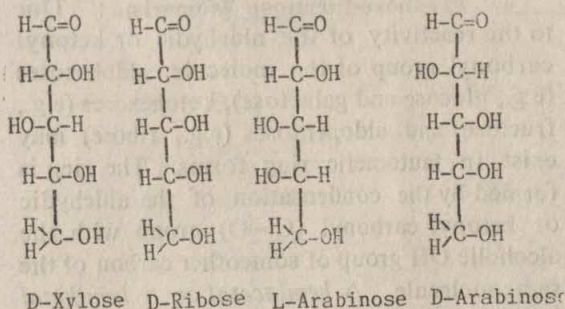


Fig. 1.4. Stereoisomerism in some pentoses.

D-glyceraldehyde (Fig. 1.3). The *L* isomer is its mirror image, with the groups of its penultimate carbon oriented like those of the middle carbon of L-glyceraldehyde (Fig. 1.5). For most sugars, the *D* isomers alone are biologically active in eukaryotes.

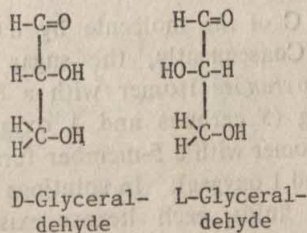


Fig. 1.5. Glyceraldehyde stereoisomers.

Epimerism is the stereoisomerism in respect of the groups of a single asymmetric C of a molecule containing more than one asymmetric C. For example, galactose and mannose are two epimers of glucose; they differ from glucose in configuration of groups on C^4 and C^2 respectively (Fig. 1.1). Ribose and xylose are epimers differing in configuration of groups on C^3 (Fig. 1.4). Similarly,

ribulose and xylulose are epimers of one another (Fig. 1.2).

As the position of groups on a single asymmetric carbon can result in two isomers, the maximum number of possible isomers is given by 2^n where n is the total number of asymmetric carbons in the molecule.

3. Optical isomerism: *Optical activity* is the capacity of a substance to rotate the plane of vibration of the rays of polarized light passing through it. It is due to the presence of asymmetric carbons in the molecule. The *dextrorotatory* or *d* isomer of an optically active substance rotates the plane of polarized light to the right; the *levorotatory* or *l* isomer rotates it to the left. This is known as optical isomerism; *d* and *l* isomers are also indicated by prefixes (+) and (−) respectively; e.g., (+)-glucose is *d*-glucose. The optical activity of a substance is the algebraic sum of the dextrorotatory and levorotatory effects of all its asymmetric carbons. If the dextrorotation caused by some asymmetric carbons of a molecule is exactly counterbalanced by the levorotation due to its remaining asymmetric carbons, the substance has no optical activity. Some optically active substances have such a *meso* isomer; e.g., *meso*-inositol. The solution of an equimolecular mixture of the *d* and *l* isomers of a substance is optically inactive (*racemic mixture* or *dl form*); but the racemic mixture can be easily resolved into *d* and *l* isomers. Monosaccharides possess optical activities and may exist as *d* and *l* isomers.

Optical rotation is usually measured by a *polarimeter* or *polariscope* (Fig. 1.6). A long hollow barrel carries at opposite ends a polarizer Nicol prism and an analyzer Nicol prism, the latter attached to a rotating circular scale. Between the two prisms are located a quartz plate for obscuring half the field of view, and a hollow polarimeter tube of known length for carrying the solution under investigation. Monochromatic light, passing through

the polarizer prism and the quartz plate, emerges as *plane polarized light* with all its rays vibrating in a single plane. It then passes to the observer through the polarimeter tube, the analyzer prism and the telescopic eyepiece lenses. The analyzer prism is initially rotated to make both halves of the field of view look equally bright and the circular scale reading is noted as the zero position. The polarimeter tube is then filled with the test solution and placed in the polarimeter. The two halves of the field of view now look unequally illuminated due to the rotation of the plane of polarized light by the solution in the tube. The analyzer prism is rotated again until both halves look equally bright again. The difference in the circular scale reading between this point and the initial zero position gives the angle of rotation of the polarized light.

Usually, only one of the optical isomers of a substance is biologically active. Naturally occurring forms of glucose, mannose, ribose, sucrose, lactose, maltose, dextrans, starch and glycogen are dextrorotatory. Natural fructose is levorotatory. Dihydroxyacetone, a triose, has no asymmetric C and no optical activity.

4. **Pyranose-furanose isomerism:** Due to the reactivity of the aldehydic or ketonyl carbonyl group of the molecule, aldohexoses (e.g., glucose and galactose), ketohexoses (e.g., fructose) and aldopentoses (e.g., ribose) may exist in tautomeric ring forms. The ring is formed by the condensation of the aldehydic or ketonyl carbonyl (C=O) group with the alcoholic OH group of some other carbon of the same molecule. A *hemiacetal* or a *hemiketal* is thereby formed in the aldose or ketose respectively, linking the carbonyl-C with

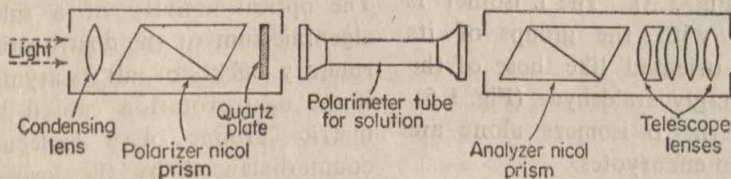


Fig. 1.6. Sketch diagram of a polarimeter.

Optical rotation of a substance in solution is expressed in terms of its *specific rotation* (α). The latter is the rotation in degrees produced at a specified temperature (25°C) in the polarized light of a standard wavelength (either sodium D-line of λ 589.3 nm or mercury green line of λ 546.1 nm) during the passage of the light through an 1 dm long column of a solution containing 1 g of the substance per ml.

$$[\alpha]_D^{25} = \frac{a}{l \times c} = \frac{100a}{l \times C}$$

where a is the observed rotation at 25°C with sodium-D light, l dm is the length of the solution column in the polarimeter tube, c is the solute concentration in g ml⁻¹, and C that in g dl⁻¹.

some other C of the molecule by a C—O—C linkage. Consequently, the sugar becomes either a *pyranose* isomer with a 6-member pyran ring (5 carbons and 1 oxygen) or a *furanose* isomer with a 5-member furan ring (4 carbons and 1 oxygen). In solutions including biological fluids, each hexose exists as an equilibrium mixture of its pyranose and furanose isomers and the open-chain form. For example, D-glucose exists as an equilibrium mixture of D-glucopyranose, D-glucofuranose and open-chain D-glucose (Fig. 1.7); of the three forms, the pyranose largely predominates in case of all aldohexoses like glucose. The pyranose can in turn show a 'chair'-'boat' isomerism with its ring existing predominantly in the 'chair' form and much less frequently in the 'boat' form (Fig. 1.8).

Aldopentoses exist in solution as equilibrium mixtures of the respective open-chain and furanose forms. Ketopentoses, trioses

5. Anomerism: In pyranose or furanose form, the aldehydic or ketonyl carbonyl-C (C^1 in aldoses and C^2 in ketoses) becomes a new

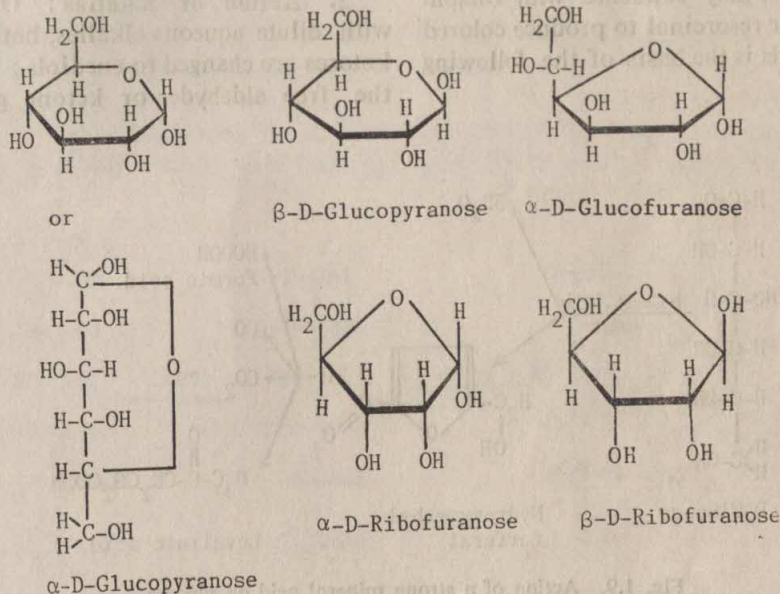


Fig. 1.7. pyranose and furanose forms of some sugars.

and tetroses cannot show pyranose-furanose isomerism. In oligosaccharides and polysac-

charides, the monosaccharide units exist in either pyranose or furanose forms only. asymmetric carbon. An additional stereoisomerism, called *anomerism*, results from the orientation of H and OH around the new asymmetric or *anomeric carbon*. Each pyranose or furanose isomer may thus exist as an equilibrium mixture of two *anomers*, α and β forms, according to the orientations of H and OH on the anomeric C; e.g., α -D-glucopyranose and β -D-glucopyranose; α -D-ribofuranose and β -D-ribofuranose (Fig. 1.7). In the α anomer, the anomeric OH group is oriented furthest from the CH_2OH group. In the β anomer, the anomeric OH is oriented close to the CH_2OH . When the open-chain form of a sugar changes in solution to pyranose or furanose form, it may produce either α or β anomer.

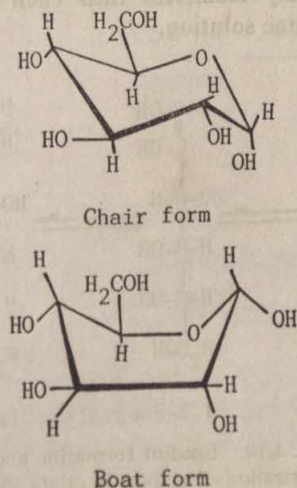


Fig. 1.8. 'Chair' and 'boat' forms of α -D-glucopyranose.

charides, the monosaccharide units exist in either pyranose or furanose forms only.

1.4 PROPERTIES OF MONOSACCHARIDES

Monosaccharides form water-soluble, colorless, odourless, sweet crystals.

1. **Action of strong acids:** On heating a sugar with strong mineral acids, the sugar loses water and forms furfural derivatives (Fig. 1.9). These may condense with α -naphthol, thymol or resorcinol to produce colored complexes. This is the basis of the following tests.

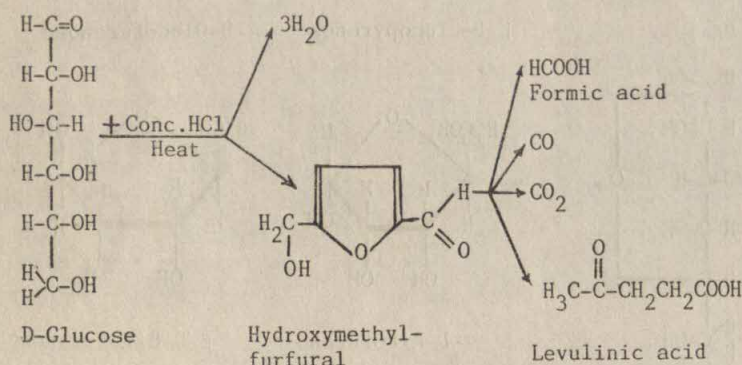


Fig. 1.9. Action of a strong mineral acid on glucose.

(a) **Molisch test:** Conc. H_2SO_4 is gently added to a mixture of the sugar solution and Molisch reagent (an alcoholic solution of α -naphthol or thymol). The heavy acid forms a separate layer below the mixed solution and a red-violet ring is produced between the layers. While all carbohydrates give this test, it is not specific for carbohydrates; it is also given by other furfural-yielding substances, aldehydes, acetone, and lactic, citric, oxalic and formic acids.

(b) **Seliwanoff's test:** A cherry-red color is produced within 30 seconds, on boiling a ketose solution (e.g., fructose) with Seliwanoff's reagent (resorcinol in dil. HCl).

(c) **Bial's test:** A green color is immediately produced on boiling a ribose solution with Bial's reagent containing HCl, orcinol and FeCl_3 . Fructose gives a red color in this test; hexuronic acids give the same green color as pentoses, but less readily.

(d) **Tollen's phloroglucinol-HCl test:** Galactose and ribose give a cherry-red color on being boiled with HCl and phloroglucinol.

2. **Action of alkalis:** On treatment with dilute aqueous alkalis, both aldoses and ketoses are changed to enediols; this involves the free aldehyde or ketone group in the

molecule (Fig. 1.10). Through the formation of a common 1,2-enediol, glucose, fructose and mannose may isomerize into each other in a dilute alkaline solution.

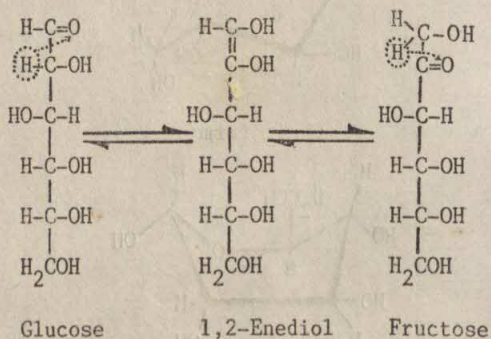


Fig. 1.10. Enediol formation and isomerization of hexoses in dilute alkali.

The enediols are good reducing agents and form the basis of reducing actions of sugars in alkaline medium.

3. Oxidation : (a) *Mild oxidizing agents* such as sodium hypoiodite and bromine water (HOBr) oxidize an aldose to an *aldonic acid* like gluconic acid, by oxidizing only the aldehyde group (Fig. 1.11). (b) *Strong oxidizing agents* like nitric acid oxidize aldoses at both aldehyde and primary alcohol groups to form *saccharic acids*. Galactose is oxidized by HNO_3 to galactaric (mucic) acid forming

group in place of the terminal CH_2OH group (Fig. 1.11). Hexuronic acids, thus formed from hexoses, give following tests :

(i) *Bial's test* : Hexuronic acids produce a green colour on boiling with Bial's reagent, but less readily than pentoses.

(ii) *Tollen's naphthoresorcinol test* : Both glucuronic acid and ribose impart a red-violet color to an ether extract of a boiled mixture

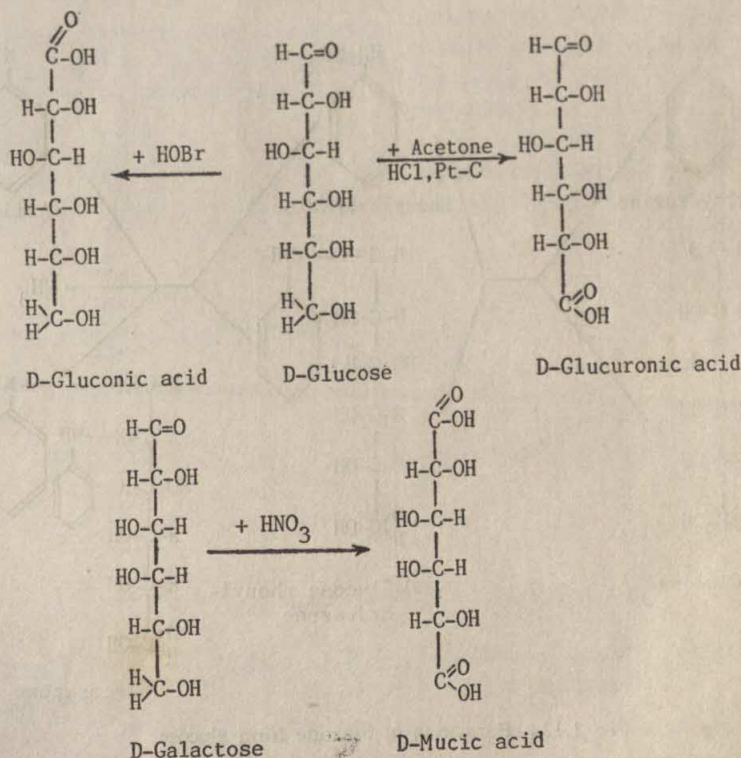


Fig. 1.11. Oxidations of aldoses.

characteristic microscopic crystals (*mucic acid test* for galactose). (c) *Platinum-carbon catalyst* (Pt-C) may oxidize only the terminal CH_2OH group of an isopropylidene derivative of an aldose to a COOH group. On hydrolysis with dil. HCl , the product yields a *uronic acid* like glucuronic acid. Uronic acids possess the unchanged C^1 -aldehyde group, but a COOH

of conc. HCl , alcoholic naphthoresorcinol and the sugar.

4. *Reduction* : Sugar alcohols (e.g., sorbitol) are produced by reducing the aldehyde or ketone group of a monosaccharide with sodium borohydride, sodium amalgam, or hydrogen at high pressure with Ni catalyst.

5. Reaction with phenylhydrazine and osazone test : The presence of a free carbonyl (aldehyde or ketone) group in the molecule is essential for this reaction. On being heated to 100°C with phenylhydrazine in acetic acid, a monosaccharide (or a reducing disaccharide) is first changed to a sugar phenylhydrazone by the reaction of the reagent with the free carbonyl (aldehyde or ketone) group; the product reacts with more reagent to give a

and melting points. So, glucose, lactose and maltose can be distinguished from each other by their osazone crystals. Nonreducing sugars like the disaccharide sucrose cannot form osazones due to the absence of a free aldehydic or ketonyl carbonyl group in them.

6. Reducing action : Reducing action of sugars depends on the free carbonyl (aldehyde or ketone) group in their molecules. In alkaline medium, a sugar with such a free

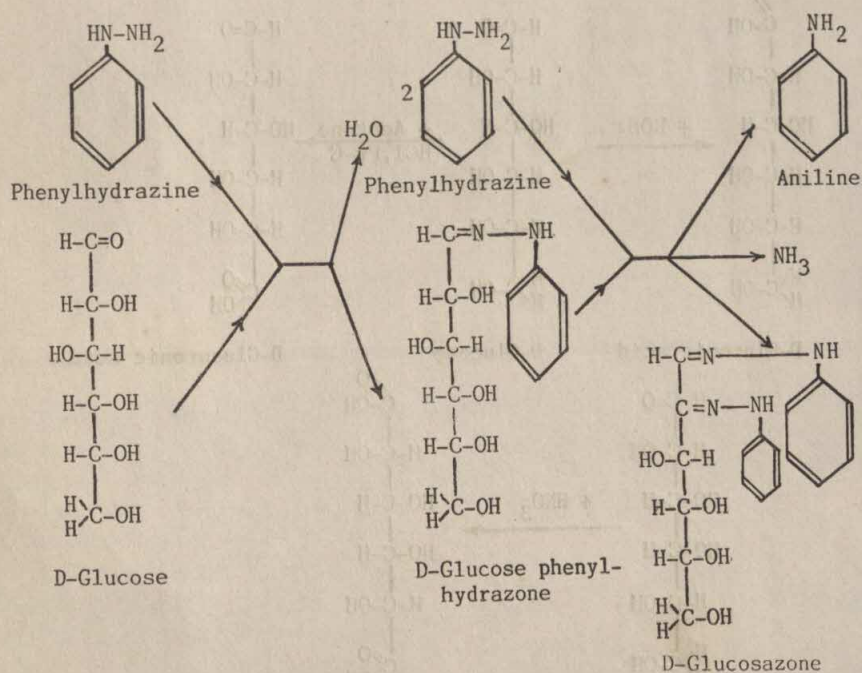


Fig. 1.12. Formation of osazone from glucose.

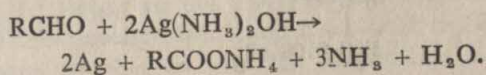
sugar osazone (Fig. 1.12). An osazone forms characteristic yellow or orange microscopic crystals of specific melting points. This *osazone test* distinguishes between different sugars. Glucose, fructose and mannose form identical osazone molecules. So, osazones of these three sugars form crystals of identical forms and melting points. But the osazone crystals of glucose and of the reducing disaccharides, lactose and maltose, differ in forms

carbonyl group forms enediols. Enediols then reduce Cu^{2+} , Ag^+ , Fe^{3+} and Bi^{3+} , and themselves get oxidized to sugar acids. Such sugars are called *reducing sugars*; e.g., glucose and fructose. Sugars having no free carbonyl group cannot form enediols and are consequently nonreducing; e.g., the disaccharides sucrose and trehalose.

(a) *Benedict's test and Fehling's test* : The reagents contain Cu^{2+} (cupric sulfate),

an alkali (Na_2CO_3 in Benedict's reagent and KOH in Fehling's reagent), and either sodium citrate (Benedict's reagent) or sodium-potassium tartrate (Fehling's reagent) to keep Cu^{2+} ions in solution. On being heated with any of these two reagents, the reducing sugar forms enediol in the alkaline medium; the enediol then reduces Cu^{2+} to Cu^+ which precipitates as yellow CuOH or red Cu_2O (Fig. 1.13).

(b) *Tollen's mirror test*: Heated with ammoniacal silver nitrate solution, a reducing sugar changes to enediol. The latter reduces Ag^+ to metallic silver, precipitating it in a shining mirror:



result from the configuration of H and OH groups of the asymmetric carbonyl-C of the tautomeric pyranose or furanose form of the sugar (Fig. 1.7). α and β anomers of a sugar have different specific rotations. In solution, each anomer gradually changes into the other until an equilibrium mixture of the two is attained. This causes a gradual change in the optical rotation until it stabilizes as the resultant optical rotation of the two anomers in the equilibrium mixture. For example, an aqueous solution of glucose shows initially a dextrorotation of $+18.7^\circ$ or $+112.2^\circ$, according as the glucose has been earlier crystallized from hot pyridine or cold ethanol and consists of β and α anomers respectively. But on standing, the specific rotation gradually changes to $+52.7^\circ$, because an equilibrium

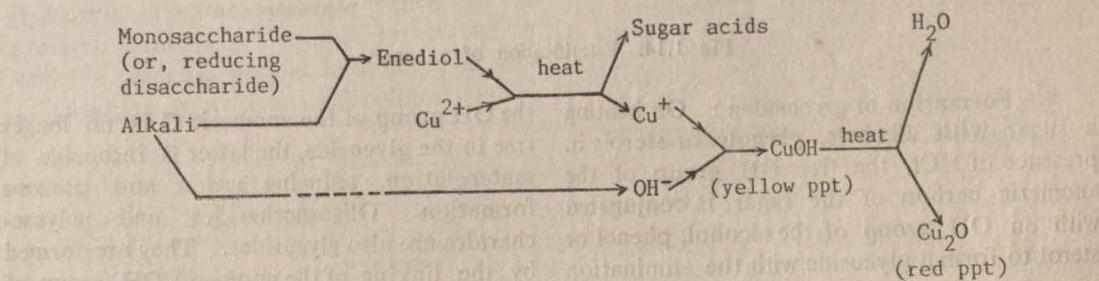
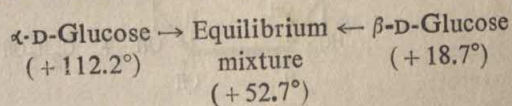


Fig. 1.13. Reduction of Cu^{2+} by reducing sugars.

(c) *Barfoed's test*: The reagent contains cupric acetate and dilute acetic or lactic acid. On being heated with the reagent, monosaccharides reduce Cu^{2+} to Cu^+ which precipitates as red Cu_2O . Reducing disaccharides reduce Barfoed's reagent much more slowly. So, the test may distinguish monosaccharides from reducing disaccharides.

7. *Mutarotation*: It is the change in optical activity of a fresh aqueous solution of a reducing sugar until its optical rotation attains a stable equilibrium. Mutarotation results from the existence of reducing sugars in α and β anomeric forms. These forms

mixture of α and β anomers is ultimately attained in the solution.



Both pyranose and furanose forms of reducing sugars exhibit mutarotation for similar reasons. But nonreducing sugars (e.g., the disaccharide sucrose) cannot show mutarotation due to the absence of the free anomeric OH group.

8. *Formation of esters*: The alcoholic groups of monosaccharides react with acid

anhydrides and acyl chlorides of acetic, phosphoric and sulfuric acids to form esters (Fig. 1.14). Glucose 1-phosphate and glucose 6-phosphate are biologically important phosphate esters of glucose, carrying the phosphate on their C^1 and C^6 respectively.

is called a *glycosidic linkage* or *glycoside bond*. It is called an α - or a β -glycosidic linkage according as it is connected with the anomeric carbon of an α or β sugar.

Only reducing sugars with a free OH group on the anomeric C can form glycosides. As

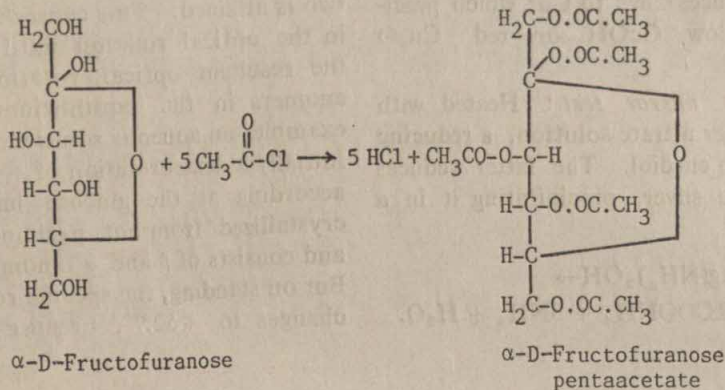


Fig. 1.14. Esterification of a sugar.

9. Formation of glycosides : On heating a sugar with alcohols, phenols or sterols in presence of HCl , the free OH group of the anomeric carbon of the sugar is conjugated with an OH group of the alcohol, phenol or sterol to form a glycoside with the elimination

of the OH group of the anomeric C is no longer free in the glycoside, the latter is incapable of mutarotation, reducing action and osazone formation. Oligosaccharides and polysaccharides are also glycosides. They are formed by the linking of the anomeric OH group of

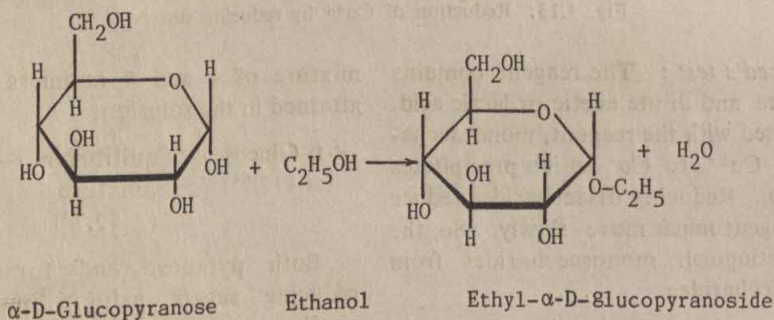


Fig. 1.15. Formation of glycoside.

of H_2O (Fig. 1.15). The covalent bond joining the anomeric carbon of a carbohydrate with the alcoholic OH group of some other molecule

one monosaccharide with an OH group of the next monosaccharide molecule by glycosidic linkage (Figs. 1.21 and 1.22). However, a

disaccharide like lactose or maltose, formed by the glycosidic linkage between the anomeric C of one monosaccharide and an OH group of another monosaccharide, may still form osazone and may exert reducing action, provided its second monosaccharide residue carries a free OH group on its anomeric C.

1.5 MODIFIED FORMS OF MONOSACCHARIDES

Some modified forms of monosaccharides occur as constituents of many molecules like nucleotides, nucleic acids, oligosaccharides, polysaccharides, gangliosides, proteoglycans and glycoproteins.

1. Deoxy sugars : They are reduced forms of monosaccharides. In them, an alcoholic OH group attached to a specific carbon has been deprived of its oxygen. Of them, L-fucose (6-deoxy-L-galactose) occurs mainly in the pyranose form (Fig. 1.16). It is one of the very few L-sugars present in eukaryotic cells. L-Fucose is a constituent of the oligosaccharide

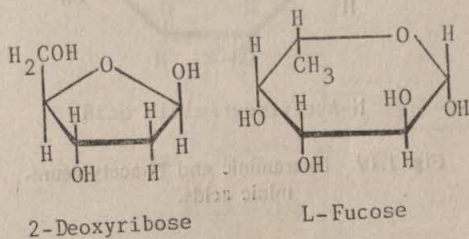


Fig. 1.16. Some deoxy sugars.

part of many membrane glycoproteins. 2-Deoxyribose, a reduced form of ribose, occurs in the β furanose form in DNA and deoxyribonucleotide molecules. Deoxyribose gives a blue color with glacial acetic acid, H_2SO_4 and Fe^{2+} (*Kiliani's test*), or when heated with diphenylamine (*Dische test*).

2. Amino sugars : They are basic modifications of monosaccharides. In them, an alcoholic OH group is replaced by the basic amino group ; e.g., glucosamine and galactosamine. Often the NH_2 group is acylated or

sulfated, and one or more OH groups are also sometimes sulfated ; e.g., N-acetylgalactosamine 6-O-sulfate, N-acetylglucosamine and glucosamine N-sulfate 6-O-sulfate (Fig. 1.17). Such C_6 amino sugars or hexosamines occur in α or β pyranose forms in many structural polysaccharides. Hexosamines have reducing properties and may form phenylhydrazones with phenylhydrazine, but not osazones, because the $\text{C}^2\text{-OH}$ group has been replaced by the NH_2 group.

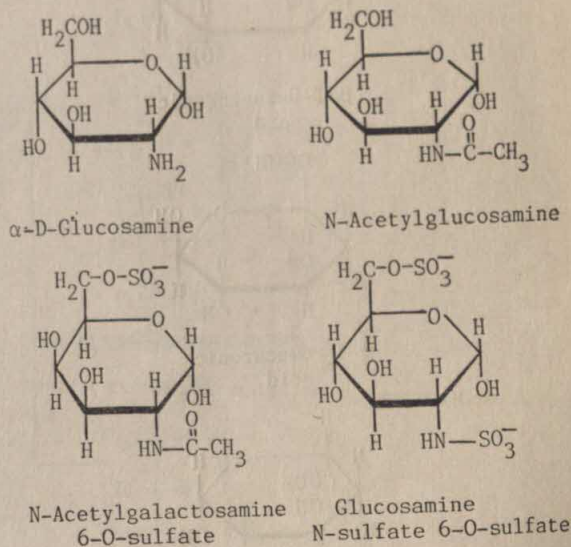


Fig. 1.17. Some amino sugars.

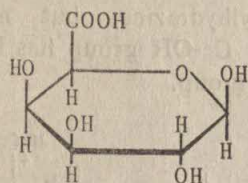
3. Uronic acids : These are acidic modifications of monosaccharides, produced by oxidation of the CH_2OH group to COOH group ; e.g., glucuronic and galacturonic acids (Fig. 1.18). Such C_6 uronic acids or hexuronic acids and their sulfated forms (e.g., iduronate 2-O-sulfate) occur in α or β pyranose forms in many structural polysaccharides (Fig. 1.28).

4. Sialic acids : These acidic modifications of monosaccharides are N-acyl derivatives of a C_6 amino sugar acid called neuraminic acid (Fig. 1.19). Examples are N-acetylneuraminic acid (NANA) and N-glycolylneuraminic acid. They are constituents of

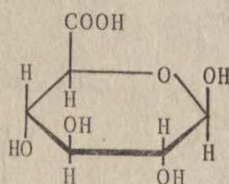
many structural polysaccharides, membrane oligosaccharides and gangliosides.

1.6 DISACCHARIDES

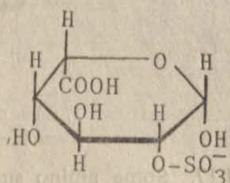
These are compound carbohydrates, each made of two monosaccharide molecules joined by a glycosidic linkage.



β -D-Galacturonic acid



β -D-Glucuronic acid

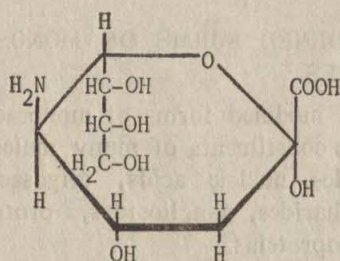


α -L-Iduronate
2-O-Sulfate

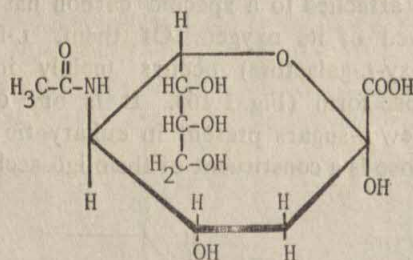
Fig. 1.18. Some hexuronic acids.

Sucrose: It is a disaccharide present in sugar beet, sugar cane juice, and ripe fruits like pineapple. Sucrose (m.p. 180°C) is composed of one molecule each of glucose and fructose; the hemiacetal $\text{C}^1\text{-OH}$ of α -D-glucopyranose is joined by an α, β -1,2-glycosidic linkage with the hemiketal $\text{C}^2\text{-OH}$ of β -D-fructofuranose (Fig. 1.20). So, there is no free anomeric OH group (potential aldehyde or ketone group) in sucrose. Hence, the latter is a nonreducing sugar. It cannot reduce

Benedict's solution, shows no mutarotation and forms no osazone. On hydrolysis with sucrase or by boiling with dil. HCl, dextrorotatory sucrose ($+66.5^{\circ}$) first yields α -D-glucopyranose and β -D-fructofuranose, both dextrorotatory. But most of the latter changes



Neuraminic acid



N-Acetylneuraminic acid

Fig. 1.19. Neuraminic and N-acetylneuraminic acids.

spontaneously to levorotatory β -D-fructopyranose while α -D-glucopyranose changes partly to β -D-glucopyranose. As the levorotatory effect of β -D-fructopyranose (-92°) exceeds the dextrorotatory effect ($+52.7^{\circ}$) of the equilibrium mixture of β -D-glucopyranose and α -D-glucopyranose, the mixture of the hydrolyzed products finally shows levorotation. Because of this change from dextrorotation to levorotation, the hydrolysis of sucrose is called the *inversion of sucrose*. Sucrase is also known as *invertase* and the mixture of glucose and fructose, obtained by hydrolyzing sucrose, is called the *invert sugar*.

Lactose: Lactose (m.p. 203°C) or milk sugar is a dextrorotatory disaccharide, composed of one molecule each of galactose and glucose. A β -1,4-glycosidic linkage joins the hemiacetal $\text{C}^1\text{-OH}$ group of β -D-galactopyranose with the $\text{C}^4\text{-OH}$ group of D-glucopyranose (Fig. 1.21). According to the α - or β -D-glucopyranose residue in its molecule,

solution, and forms characteristic lactosazone crystals which distinguish it from maltose and monosaccharides. But lactose is much slower than monosaccharides in reducing *Barfoed's reagent*.

Feaons' test: An intense red color develops when a mixture of lactose, methylamine hydrochloride solution and NaOH

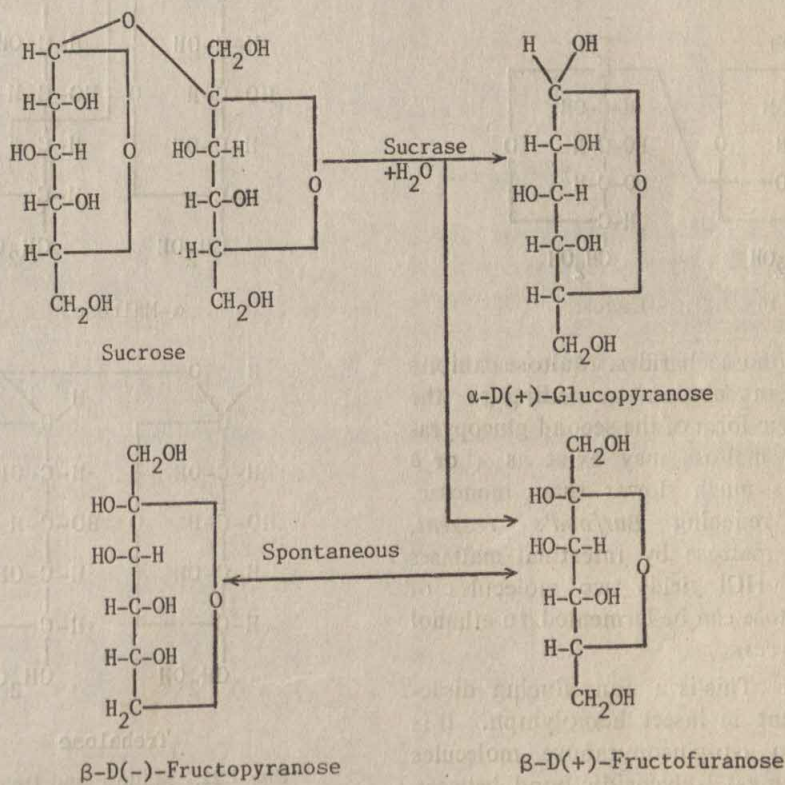


Fig. 1.20. Inversion of sucrose.

lactose may exist in α or β form. Lactose is hydrolyzed by intestinal lactase or by boiling with dilute mineral acids; this yields an equimolecular mixture of glucose and galactose.

In lactose, the hemiacetal $\text{C}^1\text{-OH}$ of the glucose residue remains free. So, lactose is a reducing sugar, shows mutarotation in

solution is heated in a waterbath at 56°C for $\frac{1}{2}$ hour and then cooled at room temperature. This test distinguishes lactose from sucrose, glucose, galactose and fructose.

Maltose: Maltose (m.p. 165°C) is a dextrorotatory disaccharide, present in malt, germinating grains, corn syrup and products of carbohydrate digestion. Its molecule is

composed of two molecules of glucose. An α -1,4-glycosidic linkage joins the C¹ of α -D-glucopyranose with the C⁴ of another glucopyranose (Fig. 1.22). As the hemiacetal C¹-OH group of the second residue remains free, maltose is a reducing sugar. It may reduce metallic ions in alkaline solutions. In osazone test, it may form characteristic maltosazone crystals that distinguish it from

glycogen. They are relatively insoluble in water, unlike monosaccharides and many small oligosaccharides; so, large amounts of such polysaccharides may be stored in cells as insoluble grains or granules, without affecting the osmotic pressure of the intracellular fluid.

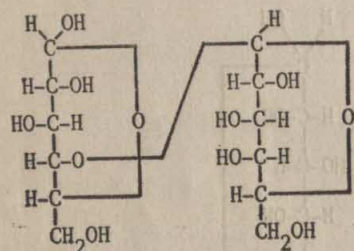


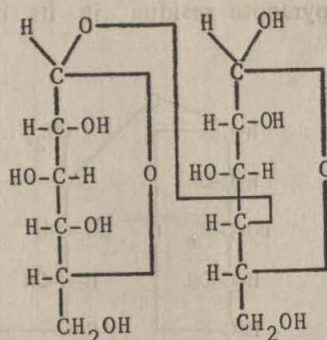
Fig. 1.21. α -Lactose.

lactose and monosaccharides. Maltose exhibits mutarotation in solution—according to the α or β anomeric form of the second glucopyranose residue, maltose may exist as α or β anomer. It is much slower than monosaccharides in reducing *Barfoed's reagent*. Hydrolysis of maltose by intestinal maltases or by dilute HCl yields two molecules of glucose. Maltose can be fermented to ethanol by *Saccharomyces*.

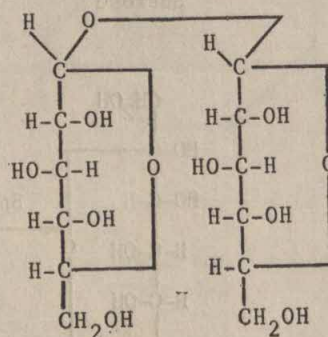
Trehalose: This is a nonreducing disaccharide, present in insect hemolymph. It is formed of two α -D-glucopyranose molecules connected by an α -1,1-glycosidic bond between their anomeric C¹ carbons. It can be hydrolyzed into two glucose molecules.

1.7 POLYSACCHARIDES

Polysaccharides have macromolecules with high molecular weights and form colloidal solutions. Some of the polysaccharides are *food-storage polysaccharides*, stored as reserve food materials in plant or animal cells. These are usually homoglycans; e.g., starch and



α -Maltose



Trehalose

Fig. 1.22. Maltose and trehalose.

Moreover, polymerization of monosaccharides into polysaccharides involves removal of many molecules of water; this reduces the bulk of the nutrient stored. Many polysaccharides are *structural polysaccharides*, forming structural components of cell wall, membranes, cell coat and extracellular matrix. They are mostly insoluble in water. A few of them are homoglycans (e.g., cellulose), but many are heteroglycans (e.g., chondroitin sulfates, dermatan

sulfate and hyaluronic acid). Some important polysaccharides are briefly described below.

Starch: Starch, $(C_6H_{10}O_5)_n$, is a plant polysaccharide stored in grains, legumes, tubers like potato, and roots like carrot. 65-85% of starch consists of branched chains of amylopectin (MW 50000-1000000) and

chains are interlinked by α -1,6-glycosidic linkages between their glucose residues to form the branched molecule (Fig. 1.24). Amylopectin molecule may carry about 300-6000 glucopyranose residues in total.

Starch is nonreducing, because most of the aldehyde groups of its glucose residues

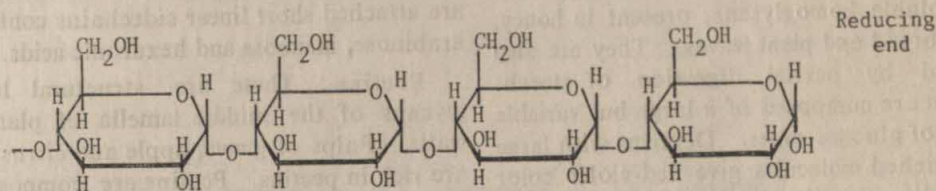


Fig. 1.23. Reducing end of amylose.

15-35% consists of unbranched α -amylose (MW 10000-100000). Both amylose and amylopectin yield many glucopyranose molecules on complete hydrolysis.

The α -amylose molecule is a helically coiled unbranched chain of 60-600 α -D-glucopyranose residues joined by α -1,4-glycosidic linkages (Fig. 1.23).

remain bound by glycoside bonds, except for the free aldehyde group of the glucose residue at one end of each linear chain. That end of the chain is called the reducing end while the other end is the nonreducing end.

Starch forms colloidal solutions by entering into water-soluble molecular aggregates called micelles. Amylose and amylopectin form

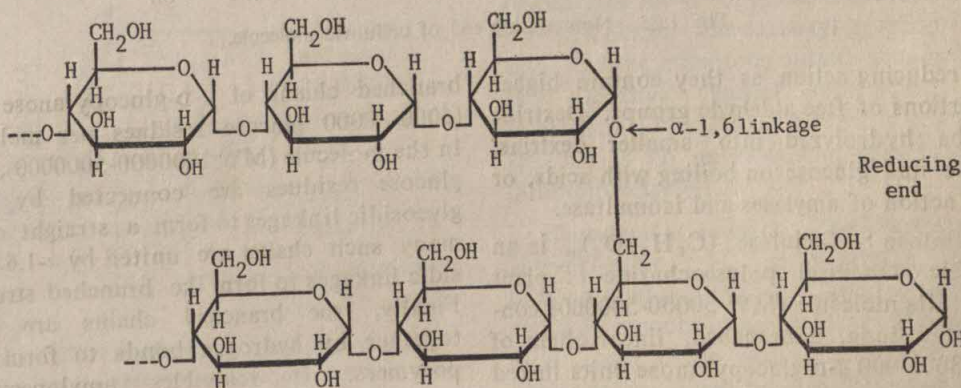


Fig. 1.24. Branching in amylopectin.

The amylopectin molecule consists of about 10-200 unbranched linear chains, each composed of 24-30 α -D-glucopyranose residues united by α -1,4-glycosidic linkages; these

blue and violet complexes with iodine, respectively; but starch gives a blue color with iodine because of more intense color of the iodine-amylose complex. The colored

complexes dissociate on heating and re-form on cooling; so, the color is lost on heating and reappears on cooling.

Starch can be hydrolyzed by boiling with dilute acids like HCl, or by amylases. Progressively smaller dextrins, maltose and ultimately glucose molecules are produced.

Dextrins: Dextrins, $(C_6H_{10}O_5)_n$, are water-soluble homoglycans, present in honey, toasted bread and plant leaves. They are also produced by partial digestion of starch. Dextrins are composed of a large but variable number of glucose units. Dextrins with large and branched molecules give red-violet color with iodine (erythrodextrins) and may not show reducing properties because of the scantiness of aldehyde groups in the large molecule. Smaller dextrins give no color with iodine (achroodextrins), are sweet and may

cellulose first into cellobiose and ultimately into glucose. Cellulose is nonreducing. It is indigestible by mammalian enzymes.

Hemicelluloses: These are structural heteroglycans forming the amorphous matrix of plant cell wall. The molecule consists of straight core-chains, made of xylose units linked by β -1,4 linkages; to these core-chains are attached short linear sidechains containing arabinose, mannose and hexuronic acids.

Pectins: These are structural heteroglycans of the middle lamella of plant cell walls. Pulp of guava, apple and citrus fruits are rich in pectins. Pectins are composed of methyl esters of galacturonic acid, galactose, arabinose and other sugars.

Glycogen: Glycogen, $(C_6H_{10}O_5)_n$, is a food-storage homoglycan, stored in liver and muscle cells. Its molecule consists of highly

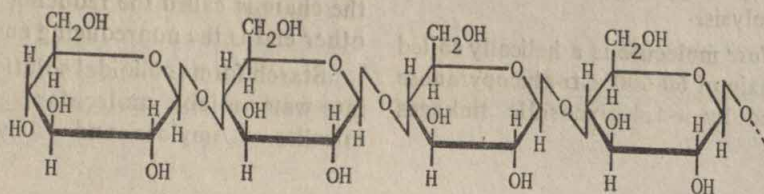


Fig. 1.25. Nonreducing end of cellulose molecule.

have reducing action as they contain higher proportions of free aldehyde groups. Dextrins may be hydrolyzed into smaller dextrins, maltose and glucose on boiling with acids, or by the action of amylases and isomaltase.

Cellulose: Cellulose, $(C_6H_{10}O_5)_n$, is an insoluble structural polysaccharide of plant fibres. Its molecule (MW 50000-500000) consists of a long, unbranched, linear chain of about 300-3000 β -D-glucopyranose units linked serially by β -1,4-glycosidic linkages (Fig. 1.25). Cellulose is the major constituent of plant cell wall. It is laid down in the form of fibrils in the amorphous matrix of the cell wall. Each cellulose fibril is composed of many bundles of parallel molecular chains of cellulose. Boiling with strong mineral acids hydrolyzes

branched chains of α -D-glucopyranose units (6000-30000 glucose residues per molecule). In the molecule (MW 1000000-5000000), 10-15 glucose residues are connected by α -1,4-glycosidic linkages to form a straight chain; many such chains are united by α -1,6-glycosidic linkages to form the branched structure. Finally, the branched chains are bound together by hydrogen bonds to form large polymers. It resembles amylopectin in structure and in giving red-violet color with iodine. Glycogen forms a colloidal solution in water, has no reducing properties and can be hydrolyzed by boiling with HCl or by amylases.

Hyaluronic acid: Hyaluronic acid, $(C_{14}H_{21}O_{11}N)_n$, is a structural heteroglycan

(MW 1000000-4000000) of animals. Its unbranched chain is composed of alternating β -D-glucuronic acid and N-acetyl- β -D-glucosamine units. C¹ of each N-acetylglucosamine is connected to C⁴ of the next glucuronic acid by β -1,4-glycosidic linkage; C¹ of glucuronic acid is joined in turn with C³ of the next N-acetyl-D-glucosamine by β -1,3 linkage (Fig. 1.26).

slippery character to the body fluids and joints.

Heparin : It is a proteoglycan formed by the binding of several heteroglycan chains to the β -OH groups of serine residues of an apoprotein. The heteroglycan (MW $\approx$ 15000) is an acid mucopolysaccharide composed of alternating units of sulfated or N-acetylated

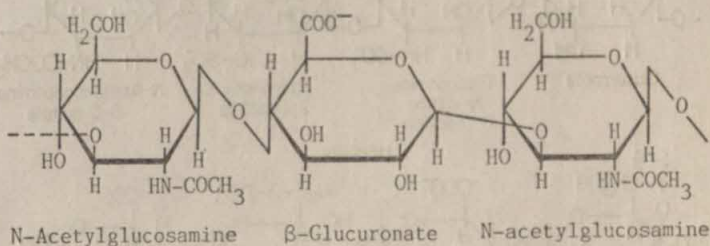


Fig. 1.26. Hyaluronic acid.

Hyaluronic acid is an acid mucopolysaccharide—free carboxyl groups in the glucuronic acid residues make it weakly acidic. It is soluble in water and forms a loose network in the solution due to the repulsion

glucosamine and sulfated or nonsulfated iduronic acid. A small number of glucuronic acid residues also occur in the chain (Fig. 1.28). The C¹ of each glucosamine is linked to the C⁴ of the next uronic acid by an α -1,4 linkage; the C¹ of each uronic acid is linked with the C⁴ of the next glucosamine by a β -1,4 linkage. The carboxyl groups of uronic acids and the numerous sulfate groups impart high negative charges and acidic nature to heparin. It is stored in mast cell granules. Heparin is an anticoagulant due to its anti-thrombin activity.

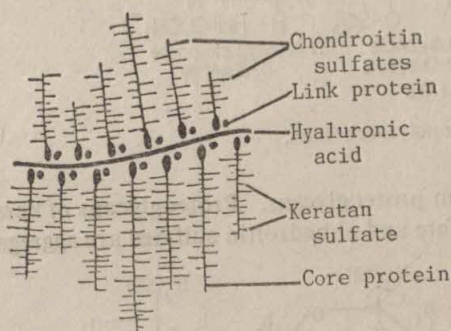


Fig. 1.27. Proteoglycan aggregate of acid mucopolysaccharides.

between its COO⁻ ions. Proteins link hyaluronic acid tightly with chondroitin sulfate and keratan sulfate to form proteoglycan aggregates in connective tissues (Fig. 1.27). Hyaluronic acid is present in the synovial fluid, umbilical cord, vitreous humor, loose connective tissue and skin. It gives a viscous

Chondroitin sulfates : These are structural heteroglycans of extracellular matrices of bones and cartilages. Chondroitin sulfates are acid mucopolysaccharides. The molecule is a linear chain made mainly of alternating glucuronic acid and sulfated N-acetylgalactosamine residues. The C¹ of each glucuronate is bound to the C³ of the next galactosamine by a β -1,3 linkage; the C¹ of the latter is bound to the next glucuronate-C⁴ by a β -1,4 linkage. The COO⁻ groups of glucuronates and the SO₃⁻ groups of sulfated N-acetylgalactosamines make the heteroglycan a polyvalent anion with

strongly acidic properties. Many chondroitin sulfate chains remain covalently bound with β -OH of serine residues of a *core protein* to form a proteoglycan. Many such proteoglycans are linked to a hyaluronic acid chain

Keratan sulfates: These structural heteroglycans occur in loose connective tissues, skin and cornea. The heteroglycan chains are bound covalently with asparagine, threonine or serine residues of apoproteins to

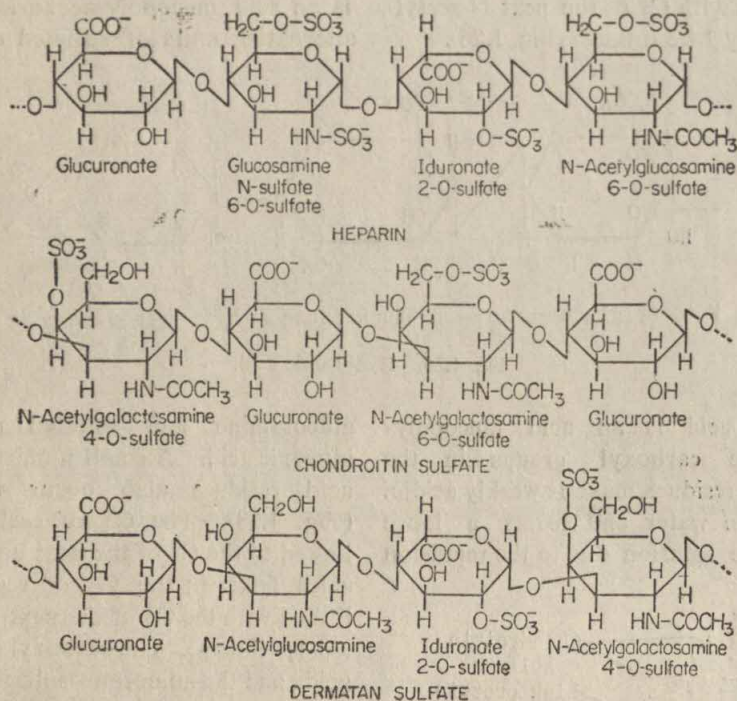


Fig. 1.28. Some acid mucopolysaccharides.

by *link protein* molecules to form a very large proteoglycan aggregate (Fig. 1.27).

Dermatan sulfates: These structural heteroglycans occur in the skin, aorta, sclera, cardiac valves and tendons. The heteroglycan chains are bound covalently with serine residues of apoproteins to form proteoglycans. The heteroglycans are acid mucopolysaccharides, made mainly of alternating residues of hexuronic acid and N-acetylhexosamine. Hexuronic acids are either L-iduronic acid 2-O-sulfate or β -D-glucuronic acid; N-acetylhexosamines are either N-acetylglucosamine or N-acetylgalactosamine 4-O-sulfate.

form proteoglycans. Proteoglycans of keratan sulfate and chondroitin sulfates are aggregated

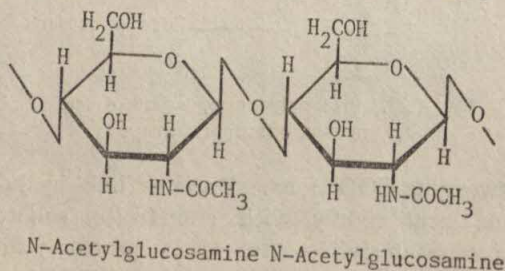


Fig. 1.29. Chitin.

in loose connective tissues by means of hyaluronic acid and core and link proteins

(Fig. 1.27). Keratan sulfates are acid mucopolysaccharides made mainly of alternating units of nonsulfated or sulfated galactose and sulfated N-acetylglucosamine. The C¹ of each galactose and the C⁴ of the next amino sugar are connected by a β -1,4 linkage while the C¹ of the amino sugar is bound to the C³ of the next galactose by a β -1,3 linkage. The SO₃⁻

groups make the heteroglycan a polyvalent anion with acidic properties.

Chitin: It is an insoluble structural polysaccharide of invertebrate exoskeletons like the insect wing and the crab shell. Its molecule is an unbranched linear chain of many N-acetylglucosamine molecules linked serially by β -1,4-glycosidic linkages (Fig. 1.29).

2. CHEMISTRY OF LIPIDS

Lipids belong to a heterogeneous class of compounds having predominantly nonpolar hydrocarbon-like molecules. They are consequently soluble in nonpolar organic solvents like benzene and chloroform, but are insoluble or only sparingly soluble in water. They form structural elements like plasma membrane, mitochondria, endoplasmic reticulum and myelin sheath. They remain stored in the adipose tissue for energy production. They are also deposited under the skin and around vital organs, for thermal insulation and thermogenesis and for acting as shock-absorbing cushions.

2.1 CLASSIFICATION OF LIPIDS

1. Simple lipids : These are esters of fatty acids with different alcohols and do not carry any other group.

(a) *Fats or glycerides or acylglycerols* : These are esters of fatty acids with glycerol, a trihydroxy alcohol. *Oils* are fats which have low melting points and remain liquid at room temperature.

(b) *Waxes* : These are solid esters of long-chain fatty acids with higher aliphatic or alicyclic monohydric alcohols ; e.g., myricyl palmitate of beeswax, and cetyl palmitate of sperm oil. *Sterol esters* also belong to this class.

2. Compound lipids : These are also fatty acid esters of different alcohols. But in addition, they carry other groups like carbohydrates, phosphate, nitrogenous bases and proteins.

(a) *Phospholipids* : These are fatty acid esters, carrying in addition a phosphate and either a nitrogenous base or an amino acid or

inositol or a second glycerol. In *phosphoglycerides*, glycerol is the alcohol ; in *sphingomyelins*, the nitrogenous alcohol sphingosine is present instead of glycerol.

(b) *Glycolipids* : These are fatty acid esters of sphingosine. But in addition to the fatty acid and sphingosine, they carry a carbohydrate. According to the nature of the carbohydrate part, glycolipids are classified further into (i) *cerebrosides* in which the carbohydrate part is either galactose or glucose, (ii) *sulfolipids or sulfatides* carrying a sulfated galactose residue, and (iii) *gangliosides* containing a branched oligosaccharide. Glycolipids are particularly rich in brain and nerve tissue.

(c) *Lipoproteins* : These are complexes of proteins and lipids. The lipid part consists of fats, phospholipids, cholesterol and its esters, and free fatty acids. They occur in cell membranes, myelin, plasma, lymph, etc.

3. Derived lipids : These are derived from simple or compound lipids by their hydrolysis.

(a) *Steroids and sterols* : Steroids have a cyclopentanophenanthrene ring, saturated to different extents and carrying different side-chains. Sterols are steroids carrying no carboxyl or carbonyl group but having one or more alcoholic hydroxyl groups.

(b) *Fatty acids* : They are hydrolytic products of fats and may be saturated or unsaturated, long-chain or short-chain, linear or cyclic. They include *prostaglandins* which are unsaturated and hydroxylated C_{20} fatty acids bearing a cyclopentane ring.

(c) *Alcohols* like glycerol, sphingosine and cetyl alcohol.

(d) *Polyisoprenoids*: They are linear or cyclic compounds made of two or more C_5 hydrocarbon units called *isoprene units*. They include carotenes, ubiquinones, dolichol, tocopherols and vitamin K.

2.2 CLASSIFICATION OF FATTY ACIDS

1. *Straight-chain fatty acids*: Fatty acids, in which the carbons are arranged linearly, belong to two subclasses:

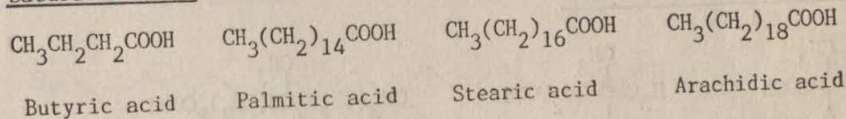
(a) *Saturated fatty acids*: There is no double bond in the carbon chain of these fatty

acid are numbered starting from the carboxyl-C taken as C_1 . *Lower fatty acids* possess ten or fewer carbons; e.g., acetic (C_2) and butyric (C_4) acids. *Higher fatty acids* have more than ten carbons; e.g., palmitic (C_{16}) and stearic (C_{18}) acids.

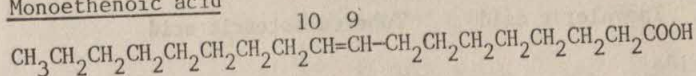
(b) *Unsaturated fatty acids*: They contain double bonds in their carbon-chains because the valencies of some carbon atoms are not saturated with hydrogen (Fig. 2.1).

(i) *Monoenoic or monoethenoic acids* carry a single double bond in the molecule, e.g., oleic

Saturated acids

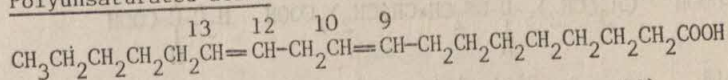


Monoethenoic acid

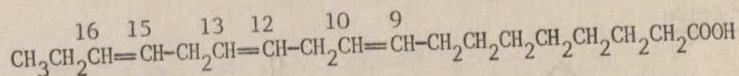


Oleic acid (*cis* 9-Octadecenoic acid)

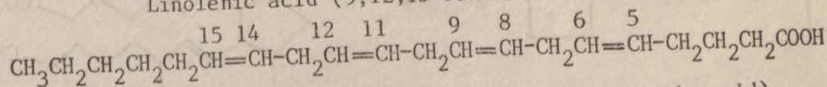
Polyunsaturated acids



Linoleic acid (9,12-Octadecadienoic acid)



Linolenic acid (9,12,15-Octadecatrienoic acid)



Arachidonic acid (5,8,11,14-Eicosatetraenoic acid)

Fig. 2.1. Some saturated and unsaturated fatty acids.

acids. They have a general formula of $C_nH_{2n+1}COOH$. (i) *Even-carbon acids* carry even number of carbons; e.g., palmitic ($C_{16}H_{33}COOH$) and stearic ($C_{18}H_{37}COOH$) acids. (ii) *Odd-carbon acids* (C_3 to C_{25}) like propionic acid (CH_3CH_2COOH) occur less frequently in the tissues. The carbons of a

acid ($C_{17}H_{33}COOH$) in animal lipids; (ii) *dienoic acids* have two double bonds, e.g., linoleic acid ($C_{18}H_{31}COOH$) in maize, soyabean, sunflower, saffola and groundnut oils and animal lipids; (iii) *trienoic acids* carry three double bonds, e.g., linolenic acid ($C_{18}H_{29}COOH$) in poppyseed oil, linseed oil



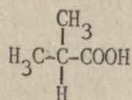
and animal lipids; (iv) *tetraenoic acids* with four double bonds, e.g., arachidonic acid ($C_{19}H_{31}COOH$) in groundnuts and animal fats. Fatty acids with more than one double bond are known collectively as *polyunsaturated* or *polyenoic acids*. The position of the double bond is indicated by the number of the carbon occupying the end of the double bond nearer to the $COOH$ group. For example, linolenic acid is a C_{18} (octadeca) acid with three double bonds at $C^9=C^{10}$, $C^{12}=C^{13}$ and $C^{15}=C^{16}$ (Fig. 2.1). So, it can be called 9,12,15-octadecatrienoic acid or $\Delta^{9,12,15}$ -octadecatrienoic acid.

2. **Branched-chain fatty acids:** These are less abundant than straight-chain acids in animal and plant lipids; e.g., isovaleric acid (Fig. 2.2).

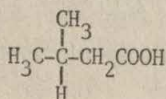
3. **Substituted fatty acids:** In hydroxy fatty acids and methyl fatty acids, one or more of the hydrogen atoms have been replaced by hydroxyl and methyl groups, respectively. They may be saturated or unsaturated; e.g., lactic acid of blood, cerebronic and oxynervonic acids of brain glycolipids, ricinoleic acid of castor oil (Fig. 2.2).

4. **Cyclic fatty acids:** Fatty acids bearing cyclic groups are present in some

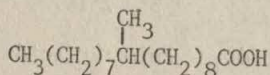
Branched chain fatty acids



Isobutyric acid

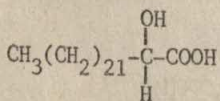


Isovaleric acid

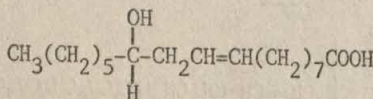


Tuberculostearic acid

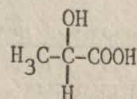
Hydroxy fatty acids



Cerebronic acid

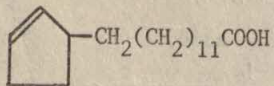


Ricinoleic acid

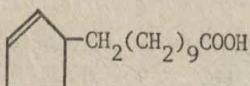


Lactic acid

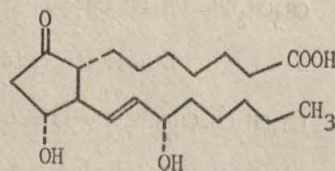
Cyclic fatty acids



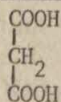
Chaulmoogric acid



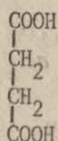
Hydnocarpic acid

Prostaglandin E_1

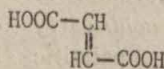
Dicarboxylic acids



Malonic acid



Succinic acid



Fumaric acid



Oxalic acid

Fig. 2.2. Some branched-chain, cyclic, substituted and dicarboxylic fatty acids.

bacterial and seed lipids; e.g., chaulmoogric acid of chaulmoogra seed. *Prostaglandins*, present in semen, blood and many tissues, belong to this class. They are unsaturated, hydroxy-substituted, cyclic C_{20} acids with a cyclopentane ring (Fig. 2.2).

5. Dicarboxylic acids: They bear two carboxyl groups; e.g., oxalic acid of spinach, succinic and fumaric acids produced in Krebs' cycle (Fig. 2.2).

2.3 ISOMERISM OF UNSATURATED FATTY ACIDS

Unsaturated fatty acids show two types of isomerism, geometric and positional.

The *cis* configuration at a double bond produces about 30° bending of the molecular chain at that double bond. The chain shows as many bendings as the number of its double bonds in *cis* configuration (Fig. 2.3). These bendings prevent the close-packing of the molecular chains of *cis* fatty acids. They consequently reduce the strength of hydrophobic bonds between the hydrocarbon chains of *cis* fatty acids. In contrast, in a *trans* isomer, the relevant radicals or groups are on the opposite sides of the axis of the double bond. Hence, its molecular chain shows very little bending at the double bond and sterically resembles the saturated fatty

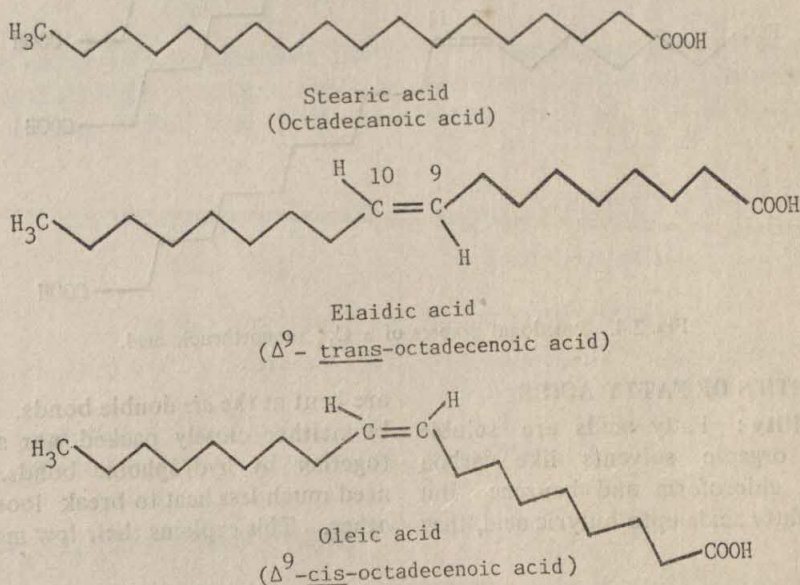


Fig. 2.3. Bendings of molecular chains in *cis* and *trans* fatty acid isomers, compared to the saturated stearic acid.

1. Geometric or *cis-trans* isomerism: This results from the restricted rotation of radicals around the axis of the double bond. If the radicals or groups, held by the carbons at the two ends of the double bond, are situated on the same side of the axis of the double bond, the compound is a *cis*

acid with no bending of its chain (Fig. 2.3). A polyunsaturated fatty acid may have a large number of geometric isomers depending on the *cis* or *trans* configurations at its numerous double bonds. Compared to the *trans* isomers, *cis* fatty acids are far more reactive, but less stable. Oleic acid (Δ^9 -*cis*-

octadecenoic acid) and elaidic acid (Δ^9 -*trans*-octadecenoic acid) are geometric isomers of one another (Fig. 2.3).

2. Positional isomerism: This results from variations in the locations of double bonds in the molecule. Different isomers of an acid possess the same number of double bonds, but at different positions in the molecular chain (Fig. 2.4).

2. Melting point: Melting point falls with the reduction in the chain-length of saturated acids and with the rise in unsaturation. C_{10} or higher saturated acids are solid at room temperature. The melting point of an even-C saturated acid is higher than those of the odd-C acids longer or shorter by a single carbon. Molecular chains of natural *cis* forms of unsaturated fatty acids

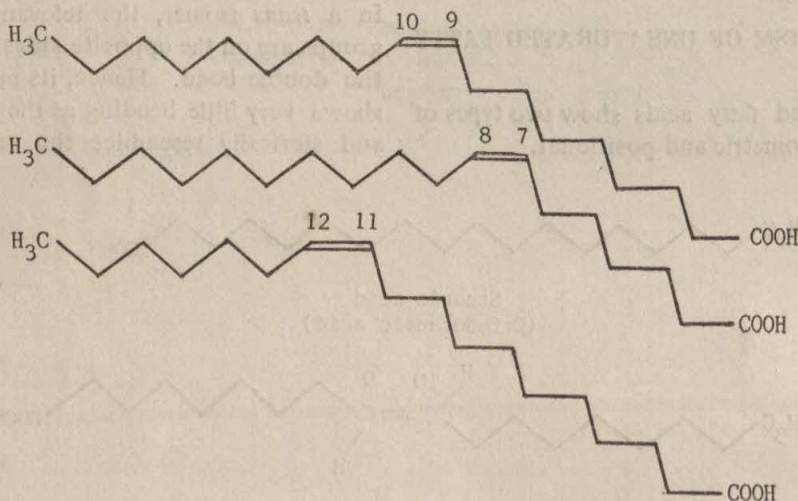


Fig. 2.4. Positional isomers of a C_{18} monoethenoic acid.

2.4 PROPERTIES OF FATTY ACIDS

1. Solubility: Fatty acids are soluble in nonpolar organic solvents like carbon tetrachloride, chloroform and benzene. But except lower fatty acids upto butyric acid, they

are bent at the *cis* double bonds. So, they can be neither closely packed nor strongly held together by hydrophobic bonds. They thus need much less heat to break loose from each other. This explains their low melting points.

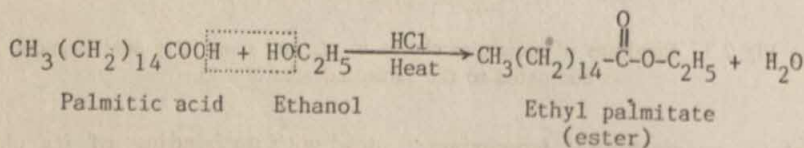


Fig. 2.5. Esterification of a fatty acid.

are not much soluble in water. Solubility decreases with the fall in temperature and the rise in chain-length of the acid, but increases with unsaturation.

3. Steam volatility: Short-chain fatty acids upto capric acid (C_{10}) can be evaporated by passing steam through their solutions. But increase in chain-length reduces steam

volatility. So, higher fatty acids are not steam-volatile.

4. Ester formation: Fatty acids react with alcohols to form esters. Triglycerides or

water-soluble than those of saturated acids. Potassium soap of an acid is more water-soluble and softer than the sodium soap; calcium and magnesium soaps are far less soluble.

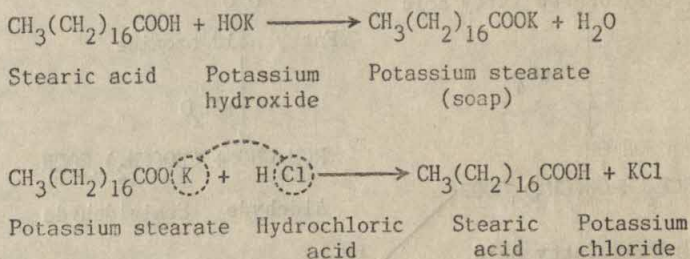


Fig. 2.6. Formation and decomposition of soap.

triacylglycerol esters can be synthesized from glycerol and acyl chloride. An ester may be prepared by heating a fatty acid with an

6. Hydrogenation: An unsaturated fatty acid or its ester accepts hydrogen at the double bonds to form a saturated or less

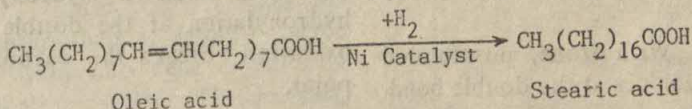


Fig. 2.7. Hydrogenation of an unsaturated fatty acid.

alcohol in presence of anhydrous HCl (Fig. 2.5).

5. Soap formation: Soaps are metallic salts of fatty acids. Soaps are formed by

unsaturated acid, when exposed to hydrogen at high pressure and temperature (150-200°C) in presence of nickel or platinum catalyst (Fig. 2.7).

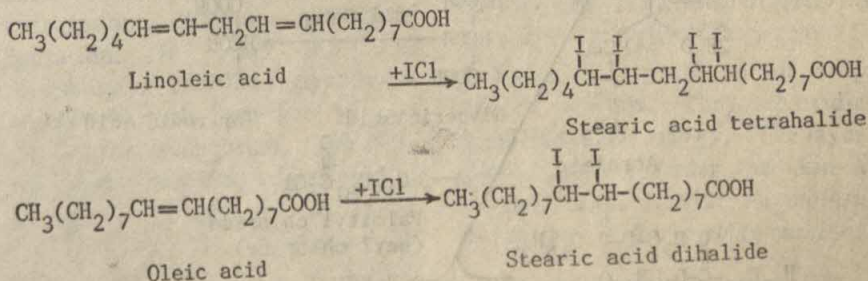


Fig. 2.8. Halogenation of unsaturated fatty acids.

adding alkalis to fatty acids (Fig. 2.6). Soaps of unsaturated fatty acids are softer and more

7. Halogenation: An unsaturated fatty acid absorbs halogens like chlorine and iodine

2.5 GLYCEROL

Glycerol is a trihydroxy alcohol. It is a derived lipid, obtained by hydrolyzing fats with water at high temperature and pressure. It is a sweet, water-soluble, ethanol-soluble, viscous liquid (sp. gr. 1.26), insoluble in non-polar fat-solvents. It forms esters with fatty and phosphoric acids. *Triglyceride esters* or *triacylglycerols* may be produced from glycerol and acyl chloride. Bromine water or H_2O_2 oxidizes glycerol to either of the trioses, glyceraldehyde and dihydroxyacetone. Glycerol is oxidized by dil. HNO_3 to glyceric acid and then, to tartronic acid (Fig. 2.10).

Acrolein test: On being heated with KHSO_4 or P_2O_5 , glycerol is dehydrated to the unsaturated aldehyde acrolein. Its irritant vapours have a characteristic pungent odour and blacken a filter paper, soaked in ammoniacal AgNO_3 , by reducing AgNO_3 to colloidal metallic silver.

Dunstan's test: Borax solution turns red on adding phenolphthalein, because borax dissociates in solution into weak boric acid and strongly alkaline NaOH . The solution is decolorized on adding glycerol, due to the formation of stronger glyceroboric acid; heat restores the color by cleaving glyceroborate into boric acid.

2.6 SPHINGOSINE

Sphingosine or 4-sphingenine is an unsaturated, nitrogenous, dihydroxy alcohol of *trans* configuration. It occurs in some lipids such as sphingomyelins and glycolipids. It may be saturated with hydrogen to give sphinganine or dihydrosphingosine. The NH_2 group of sphingosine may bind a fatty acid by an amide linkage to produce an *acylsphingosine* or *ceramide* (Fig. 2.11). Alcoholic OH groups of sphingosine may bind with phosphoric acid by ester linkage (e.g., in sphingomyelins), or with a sugar like galactose by glycosidic linkage (e.g., in cerebrosides).

2.7 FATS

Fats are esters of fatty acids with glycerol, with no other component in the molecule. Fats are classified into: (i) *triacylglycerols* or *triglycerides* or neutral fats, having three fatty acid residues esterified to a glycerol molecule,

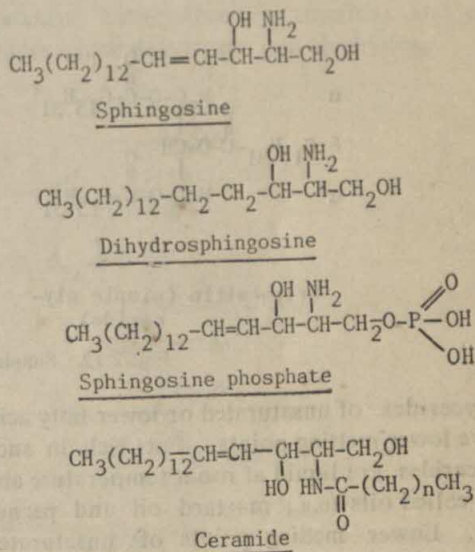


Fig. 2.11. Sphingosine and some of its derivatives.

(ii) *diacylglycerols* or *diglycerides*, containing two fatty acid residues, and (iii) *monoacylglycerols* or *monoglycerides*, with a single fatty acid combined with glycerol. Triglycerides are classified into: (a) *simple triglycerides* carrying three identical fatty acid residues, and (b) *mixed triglycerides* carrying more than one type of fatty acids (Fig. 2.12).

Fats remain stored as *fat droplets* in fat cells of animals. They are oxidized in mitochondria to give energy. The layer of fat as adipose tissue under the skin and around internal organs provide *thermoinsulation* and also act as *shock-absorbing cushions*.

2.8 PROPERTIES OF FATS

The color, odour and taste of natural fats are due to the presence of substances other

than triacylglycerols; the latter are devoid of those qualities.

1. **Melting point:** Glycerides (acylglycerols) of higher saturated fatty acids have high melting points. Fats rich in such glycerides remain solid at ordinary room temperature (e.g., hydrogenated vegetable oils).

the hydrophobic (water-repellant), nonpolar, hydrocarbon chains of their fatty acid residues. When emulsifying agents or detergents like soaps, bile salts and lecithins are added to a mixture of fat and water, a thin layer of the emulsifying agent covers the surface of each fat droplet. In each such

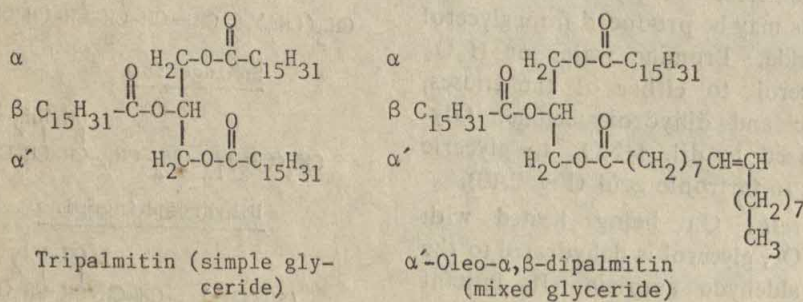


Fig. 2.12. Simple and mixed triglycerides.

Glycerides of unsaturated or lower fatty acids have lower melting points. Fats rich in such glycerides are liquid at room temperature and are called oils (e.g., mustard oil and peanut oil). Lower melting points of unsaturated fats result from the lack of close-packing and a consequent dearth of interchain hydrophobic interaction of their fatty acid tails bent at each *cis* double bond.

2. **Solubility:** All fats are soluble in nonpolar fat-solvents like chloroform, carbon

droplet, the polar or hydrophilic (water-attracting) groups of the emulsifying agent are oriented towards the surrounding water phase, but nonpolar hydrophobic groups of the agent and the nonpolar fat molecules are located in the nonaqueous interior of the droplet. Such molecular aggregates or *micelles* remain stably dispersed in water as an *emulsion*.

3. **Saponification:** Boiling with an alcoholic solution of strong metallic alkali hydro-

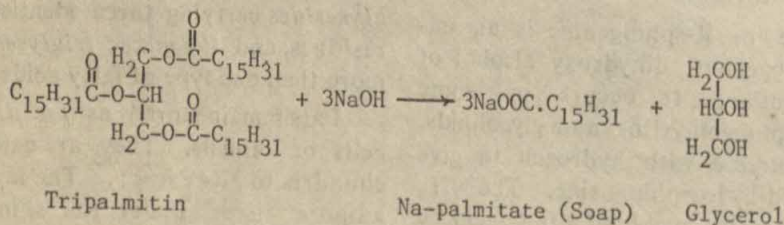


Fig. 2.13. Saponification of a simple triglyceride.

tetrachloride, ether and benzene, and in hot ethanol. Glycerides not containing hydroxy fatty acids, are soluble in petroleum ether also. Fats and lipids are insoluble in water due to

lyzes triglycerides into glycerol and fatty acids, the latter immediately forming soaps with metal ions (Fig. 2.13).

Fats, phospholipids, glycolipids and waxes

can be saponified by boiling with strong metallic alkalis. Each of these lipids contain one or more fatty acid residues which are liberated as soaps, on boiling with metallic alkalis. So, these lipids are called *saponifiable lipids*. But steroids, polyisoprenoids and higher alcohols are grouped as *unsaponifiable lipids*, because they cannot give rise to soaps.

6. **Halogenation** : On treatment with I_2 , Br_2 , IBr or ICl , unsaturated glycerides absorb two halogen atoms at each double bond.

7. **Oxidation of unsaturated glycerides** : Unsaturated glycerides may absorb oxygen and ozone at the double bonds of their fatty acids. They consequently produce enediols, epoxides, ketohydroxides, ozonides and peroxides which decompose into aldehydes.

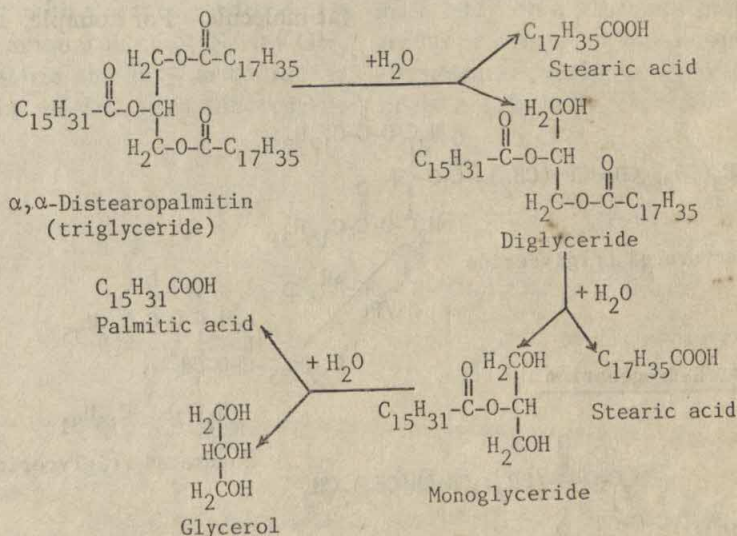


Fig. 2.14. Hydrolysis of a mixed triglyceride.

4. **Hydrolysis** : On boiling fat with water at 220°C under pressure in an autoclave, triglycerides are ultimately hydrolyzed into glycerol and fatty acids through diglyceride and monoglyceride stages (Fig. 2.14). Action of lipases or boiling with acids also hydrolyzes triglycerides.

5. **Hydrogenation** : At 150 – 220°C in presence of finely divided nickel or platinum as catalyst, glycerides of unsaturated fatty acids may be saturated with hydrogen at the double bonds of fatty acid residues ; e.g., hydrogenation of vegetable oils to 'vanaspati' or margarine (Fig. 2.15). Hydrogenation elevates the melting point.

8. **Rancidity** : Rancidity is the objectionable taste and odour of fat on standing exposed to light, moisture and air, particularly at warm temperatures. Rancidity often results from the formation of aldehydes due to the oxidation of unsaturated glycerides at their double bonds. Rancidity is also produced by the liberation of free fatty acids due to the hydrolysis of triglycerides by lipases in the natural fat, and by the microbial decomposition of fatty acids to ketones.

9. **Acetylation** : The hydroxyl groups of hydroxy fatty acid residues of a glyceride react with acetic anhydride to give an acetylated glyceride and free acetic acid (Fig. 2.16).

2.9 CHARACTERIZATION OF FATS

1. **Saponification number** : This is the mg of KOH required to saponify 1 g fat or oil. Each fatty acid residue in the triglyceride uses one KOH molecule for being saponified. So, the amount of KOH used by 1 g fat is inversely proportional to the average molecular weight of the fatty acids in the fat. Butter fat (sap. No. 210-230) carries, on average, smaller molecules of fatty acids than castor oil (sap. No. 175-180).

26-28, 80-88 and 103-110 respectively. This indicates that they carry unsaturated acids in an ascending order.

3. **Acetyl number** : It is the mg of KOH required to neutralize the acetic acid obtained by the saponification of 1 g of acetylated fat. The acetyl group is accepted only by the OH groups of hydroxy fatty acid residues in a triglyceride. So, acetyl number is a measure of the amount of hydroxy fatty acids in the fat molecule. For example, castor oil (acetyl

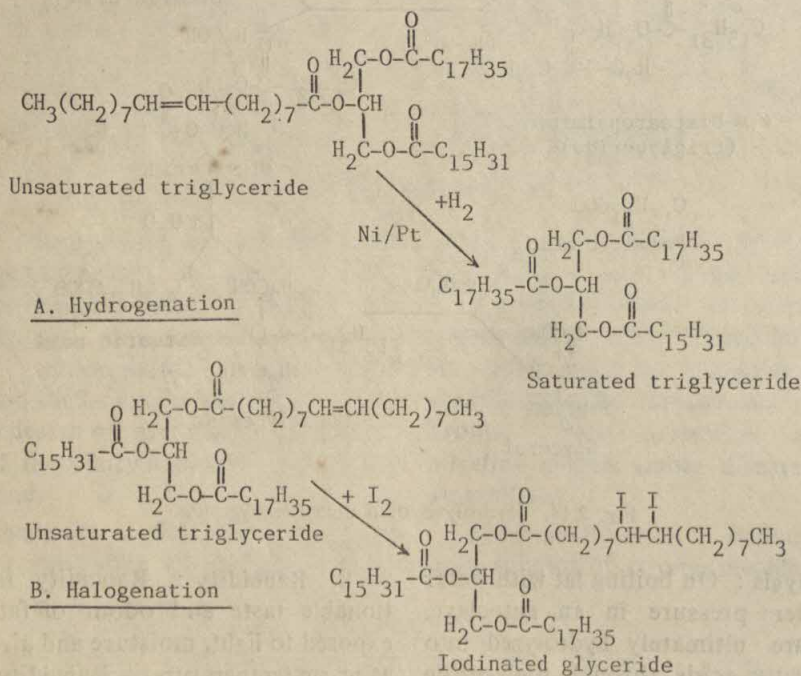


Fig. 2.15. Reactions of unsaturated triglycerides.

2. **Iodine number** : This is defined as grams of iodine absorbed by 100 g of fat. Iodine is absorbed only at the double bonds of unsaturated fatty acid residues of triglycerides. So, iodine number gives an estimate of the degree of unsaturation and the relative amounts of unsaturated fatty acids in fat molecules. Coconut oil, butter, olive oil and cottonseed oil have iodine Nos. of 6-10,

No. 146-150) contains far more hydroxy fatty acids in its glyceride molecules than olive oil (acetyl No. 10-12).

4. **Acid number** : It is the number of mg of KOH required to neutralize the free fatty acids in 1 g of fat or oil. Free fatty acids, liberated in a natural fat due to hydrolysis by lipases, may contribute towards rancidity. So, a high acid number indicates a

higher tendency for rancidity. Butter fat shows wide variations like 0.45-35.

5. **Reichert-Meissl number or volatile fatty acid number**: This gives the relative amounts of lower fatty acids in the triglyceride. A sample of fat is first saponified and then treated with mineral acids to liberate free fatty acids from the soaps. Steam is then passed through this mixture. Lower fatty acids upto capric acid (C_{10}) are volatilized and carried away with steam to condense in a receptacle. The amount in ml of N/10 KOH, required to neutralize the fatty acids in the distillate from 5 g of fat, gives the volatile

Fats rich in unsaturated or lower fatty acids have low melting points.

8. **Molecular refractivity**: Computed from refractive index, it gives an idea about the molecular structure, carbon skeleton, double bonds, and proportions of isomeric forms of fat molecules in a natural fat.

2.10 PHOSPHOLIPIDS

Phospholipids are fatty acid esters, containing in addition a phosphate group and a nitrogenous or non-nitrogenous group (e.g., choline, ethanolamine, serine and inositol). Phospholipids are broadly classified into *phospho-*

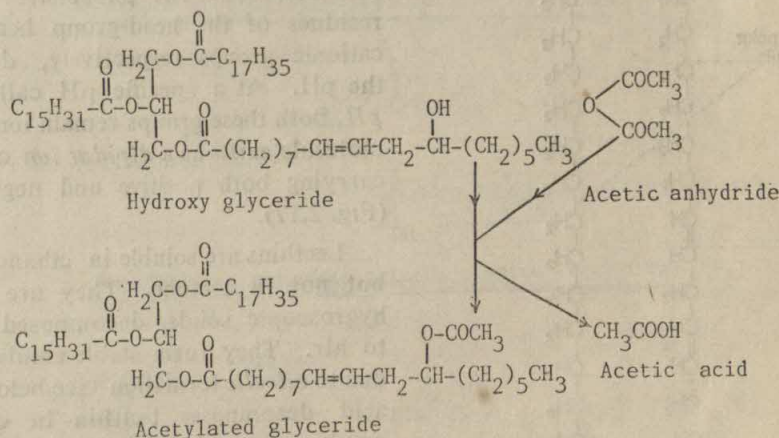


Fig. 2.16. Acetylation of a hydroxy glyceride.

fatty acid number. Butter, coconut oil and olive oil have R.M. Nos. of 20-33, 6-7.5 and 0.5-1.5 respectively. This indicates that they carry volatile fatty acids in a descending order. Agmark 'ghee' has an R.M. No. higher than 28.

6. **Polenske number**: It is the number of ml of N/10 KOH required to neutralize the non-volatile and water-insoluble fatty acids (higher than capric acid) liberated by the saponification and acidification of 5 g of a fat. Agmark 'ghee' has a Polenske number of 1-2.

7. **Melting point**: A high melting point indicates a high saturation of the triglyceride.

glycerides containing glycerol as the alcohol, and *sphingomyelins* containing the nitrogenous alcohol sphingosine instead of glycerol.

Phospholipids are *structural components*, particularly of cellular membranes. They also participate in the *absorption and transport of nonpolar lipids* like fats and sterols in the body.

Most phospholipid molecules have a charged *polar head-group* consisting of the phosphate and the nitrogenous base, and *non-polar fatty acid tails* (Fig. 2.17). Such molecules carrying both polar and nonpolar groups are called *amphipathic* molecules.

Phosphoglycerides : These are phospholipids containing glycerol as the alcohol. In most cases, the hydroxyl group of the α' carbon of glycerol is esterified with a saturated

Phosphoglycerides are derivatives of phosphatidic acid. The latter is composed of glycerol, phosphoric acid and two fatty acid molecules (Fig. 2.19).

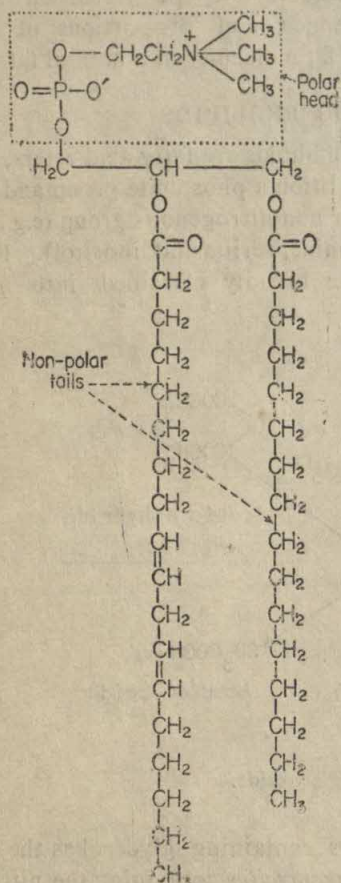


Fig. 2.17. Zwitterion form of the amphipathic phospholipid lecithin.

fatty acid while the β -hydroxyl group of glycerol is esterified with an unsaturated fatty acid. The α -hydroxyl group of glycerol is esterified to phosphocholine or phosphoethanolamine or phosphoserine, etc. (Fig. 2.18). Stearic and palmitic acids are the saturated fatty acids occurring most frequently in many classes of phosphoglycerides. Oleic, linoleic, linolenic and arachidonic acids are the most frequent unsaturated fatty acids.

1. Lecithins or phosphatidylcholines :

They are composed of glycerol, two fatty acid molecules, phosphate group and a nitrogenous base choline (Fig. 2.18). Lecithins are widely distributed in animal tissues such as brain, liver, cardiac muscle and blood.

Lecithin molecule has a charged polar head-group consisting of phosphocholine, and two nonpolar hydrocarbon tails of the fatty acid residues. The phosphate and choline residues of the head-group bear anionic and cationic groups respectively, depending on the pH. At a specific pH called *isoelectric pH*, both these groups remain ionized and the molecule exists as a *dipolar ion* or *zwitterion*, carrying both positive and negative charges (Fig. 2.17).

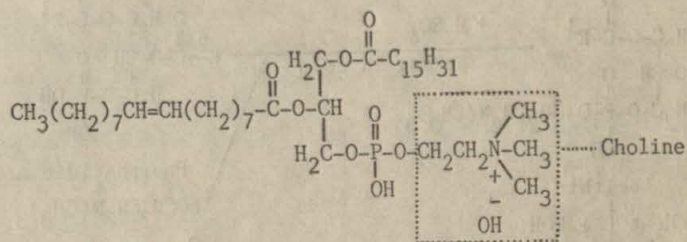
Lecithins are soluble in ethanol and ether, but not in acetone. They are waxy white hygroscopic solids, decomposed on exposure to air. They form stable emulsions in water due to *micelle* formation (see below). Sulfuric acid decomposes lecithin in cold aqueous emulsions to phosphatidic acid and choline (Fig. 2.19). Boiling with KOH or NaOH decomposes lecithin yielding soaps, glycerophosphate and choline.

2. Cephalins or phosphatidylethanolamines :

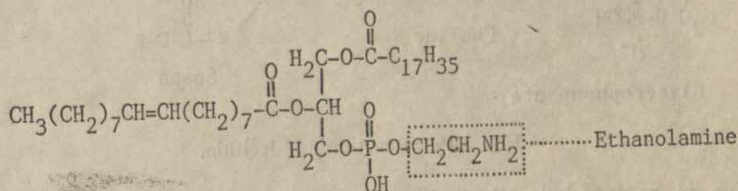
They differ from lecithins in having the nitrogenous base ethanolamine in place of choline (Fig. 2.18). They are more acidic than lecithins because the ethanolamine NH_2 group of cephalins is less basic than the quaternary ammonium group of choline in lecithins. Brain, liver, cardiac muscle and erythrocytes contain sufficient amounts of cephalins. Cephalins are hygroscopic solids, insoluble in alcohol and acetone, but soluble in ether. They can form micelles to enter into stable aqueous emulsions. At specific

isoelectric pHs , they exist as zwitterions carrying both positive and negative charges in the phosphoethanolamine head-group. The ethanolamine residue and the phosphate

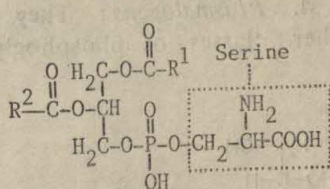
residue of the head-group ionize into cation and anion respectively. Like lecithins, cephalins are decomposed by sulfuric acid in cold and by boiling with alkali.



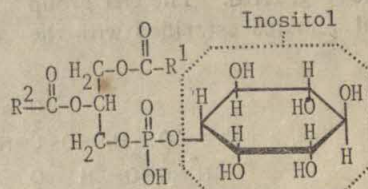
Example of a phosphatidylcholine (lecithin)



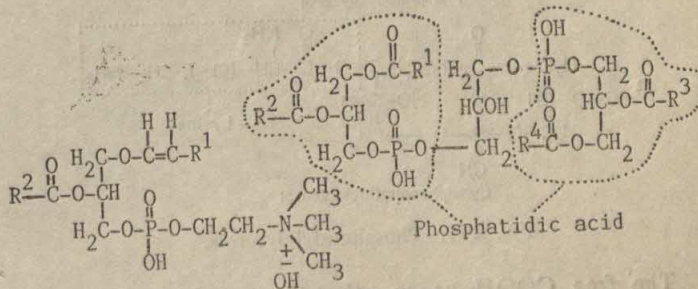
Example of a phosphatidylethanolamine (cephalin)



Phosphatidylserine



Phosphatidylinositol



Phosphatidylcholine

Cardiolipin

Fig. 2.18. Some phosphoglycerides.

3. *Phosphatidylserines, phosphatidylthreonines and phosphatidylhydroxyprolines*: They are also known as *lipoamino acids*. Choline

of the molecule carries a net negative charge in spite of the amino acid NH_3^+ group, because it contains the amino acid COO^- and the

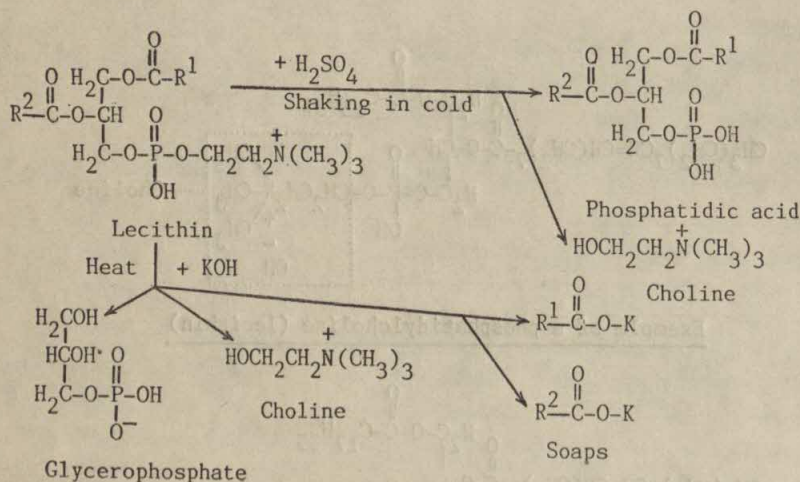


Fig. 2.19. Reactions of lecithin.

or ethanolamine is replaced in them by a hydroxy amino acid such as serine, threonine or hydroxyproline (Fig. 2.18). The OH group of the amino acid remains esterified with the

anionic group of phosphate. Lipoamino acids occur in brain and erythrocytes.

4. *Plasmalogens*: They are similar to other classes of phosphoglycerides except

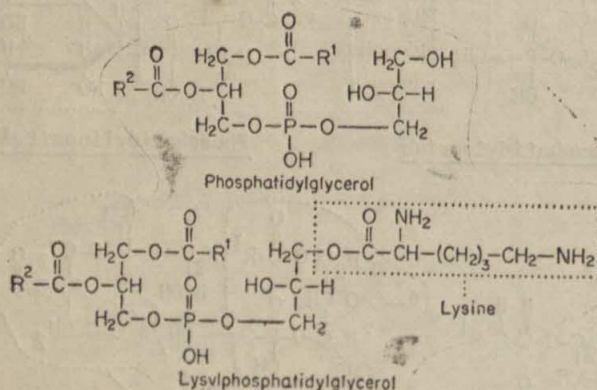


Fig. 2.20. Phosphatidylglycerols.

phosphate residue. The free COOH group of the amino acid residue makes the lipoamino acids more acidic than other phosphoglycerides. The phosphoamino head-group

that the α' carbon of glycerol is linked to an unsaturated alcohol by an ether linkage, instead of being linked to a fatty acid. Plasmalogens are classified into phosphatidyl

cholines (Fig. 2.18), phosphatidalethanolamines and phosphatidaserines according as they contain choline, ethanolamine or serine. Brain, cardiac muscle and erythrocytes contain plasmalogens.

5. *Nitrogen-free phosphoglycerides*: In them, the nitrogenous group is replaced by non-nitrogenous substances. (i) *Phosphatidylglycerols* (Fig. 2.20) resemble lecithins and cephalins in composition except that the nitrogenous base is replaced here by another

glycerol molecule esterified with the phosphoric acid. Sometimes a molecule of the amino acid lysine remains esterified with the second glycerol to form *lysylphosphatidylglycerol*. (ii) *Diphosphatidylglycerols* (Fig. 2.18) bear two molecules of phosphatidic acids joined by a molecule of glycerol (e.g., cardiolipin in cardiac mitochondria). (iii) *Lipositols* or phosphatidylinositols resemble lecithins in composition, but they contain inositol in place of choline (Fig. 2.18). Lipositols are

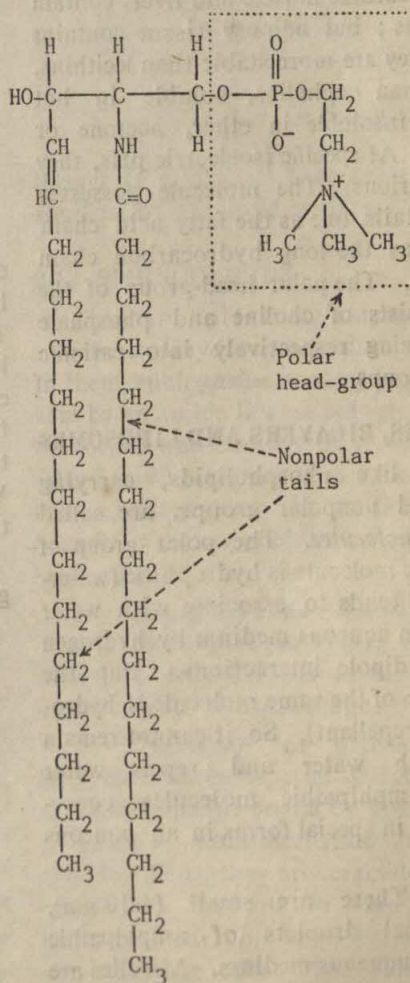
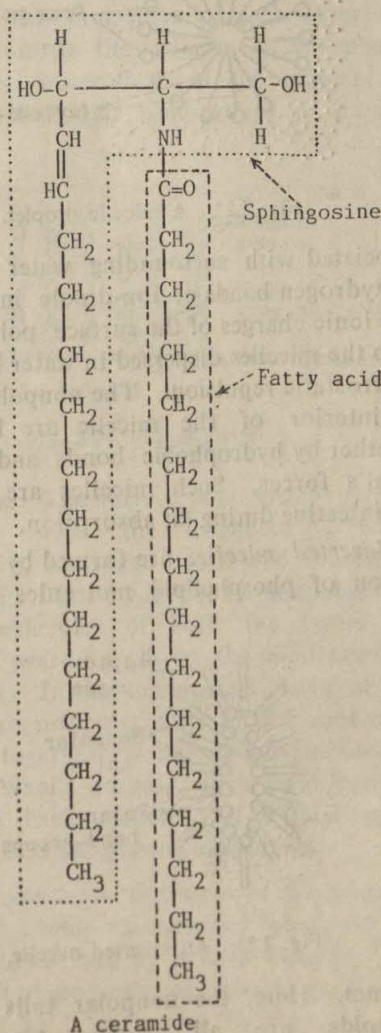


Fig. 2.21. Sphingomyelin and ceramide.

found in soyabean, cardiac muscle, brain and liver.

Sphingomyelins : They are phospholipids carrying sphingosine, dihydrosphingosine or polyunsaturated sphingosine as the alcohol in place of glycerol. The molecule carries a single fatty acid residue bound to the NH_2 group of sphingosine to form a *ceramide* residue; a phosphate is esterified to the CH_2OH group of ceramide and a choline is in turn esterified to the phosphate (Fig. 2.21). Blood, brain, cardiac muscle and liver contain sphingomyelins; but adipose tissue contains very little. They are more stable than lecithins, less acidic than cephalins, soluble in hot ethanol, but insoluble in ether, acetone or cold ethanol. At specific isoelectric pHs, they exist as zwitterions. The molecule possesses two nonpolar tails, one as the fatty acid chain and the other as the long hydrocarbon chain of sphingosine. The polar head-group of the molecule consists of choline and phosphate residues, ionizing respectively into cationic and anionic groups.

2.11 MICELLES, BILAYERS AND LIPOSOMES

Molecules like phospholipids, carrying both polar and nonpolar groups, are called *amphipathic molecules*. The polar group of an amphipathic molecule is hydrophilic (water-attracting) and tends to associate with water molecules in an aqueous medium by hydrogen bonds or ion-dipole interactions. But the nonpolar group of the same molecule is hydrophobic (water-repellant). So, it cannot remain associated with water and repels water molecules. Amphipathic molecules consequently remain in special forms in an aqueous medium.

Micelles : These are small (4-10 nm), stable, spherical droplets of amphipathic materials in an aqueous medium. Micelles are formed when the amphipathic substance occurs above a specific minimum concentration (*critical micelle concentration*). Each micelle

is an aggregate of about 50-100 amphipathic molecules such as phospholipid molecules. The polar head-groups of all the molecules are oriented towards the surrounding aqueous phase on the outer surface of the micelle. But their nonpolar tails are all hidden in the nonaqueous central part of the micelle (Fig. 2.22). The surface polar groups remain

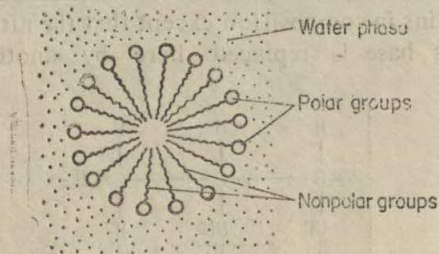


Fig. 2.22. A micelle droplet.

associated with surrounding water molecules by hydrogen bonds or ion-dipole interactions. The ionic charges of the surface polar groups keep the micelles dispersed in water by mutual electrostatic repulsion. The nonpolar tails in the interior of the micelle are held close together by hydrophobic bonds and Van der Waal's forces. Such micelles are formed in the intestine during fat absorption.

Inverted micelles are formed by the aggregation of phospholipid molecules in a lipid

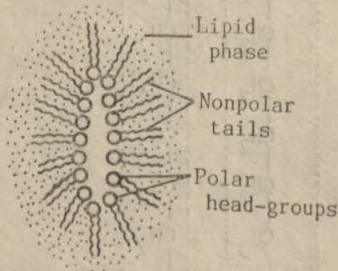


Fig. 2.23. An inverted micelle.

medium. Here, the nonpolar tails of phospholipids are all oriented towards the surrounding lipid phase on the outer surface of

the droplet. But their polar head-groups are all oriented towards the centre of the droplet where they lie hidden from the surrounding lipid medium. Inverted micelles occur along the lines of fusion between plasma membranes of adjacent cells in tight junctions (Fig. 2.23).

Bilayers: A large number of phospholipid molecules may be arranged in a *bilayer* in an aqueous medium (Fig. 2.24). This lipid bilayer consists of two layers of phospholipid molecules. The hydrophilic polar head-groups of the molecules of each layer are oriented towards the adjacent water phase. So, the two outer faces of the bilayer are formed of the polar head-groups of its two

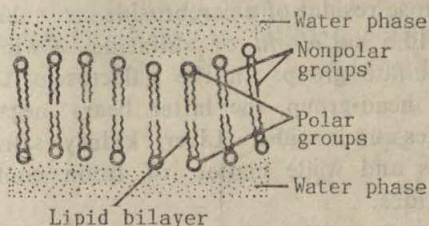


Fig. 2.24. Lipid bilayer.

lipid layers. The surface polar groups remain associated with the neighbouring water molecules by hydrogen bonds and ion-dipole interactions. The hydrophobic nonpolar tails of the molecules of both the layers are oriented inwards to form the middle core of the bilayer. In the nonaqueous phase of the middle core, nonpolar tails of the molecules are held together by hydrophobic bonds and Van der Waal's forces. Such lipid bilayers form the basic structure of cellular membranes such as the plasma membrane.

Liposomes: A liposome is a spherical vesicle enclosing an aqueous phase in its interior and covered by a lipid bilayer. The phospholipid molecules of the outer and inner layers of the bilayer have their polar head-groups on the respective surfaces of the

bilayer; but their nonpolar tails are lodged in the nonaqueous middle lamella of the

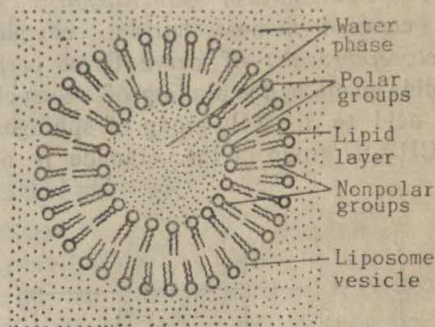


Fig. 2.25. A liposome.

bilayer (Fig. 2.25). Liposomes result from sonification of a lipid in water.

2.12 SPHINGOLIPIDS

These are lipids containing sphingosine (or sometimes dihydrosphingosine) as the alcohol. In them, sphingosine remains bound to a fatty acid by an amide linkage between the NH_2 of the former and the COOH of the latter; this fatty acid-sphingosine or acylsphingosine residue, called *ceramide*, has its CH_2OH group bound to various nonlipids to form different sphingolipids.

1. **Sphingomyelins:** These constitute one class of phospholipids (vide 2.10). In their molecules, the CH_2OH of ceramide remains esterified with phosphocholine.

2. **Glycolipids or glycosphingolipids:** In their molecules, the CH_2OH group of ceramide remains bound by glycosidic linkage to either a monosaccharide or an oligosaccharide. Thus, they are ceramide-carbohydrate complexes. Glycolipids are amphipathic molecules, carrying a polar head-group and two nonpolar tails. The polar head-group consists of the carbohydrate residue and may be charged or uncharged (Fig. 2.26). The nonpolar tails are the hydrocarbon chains of

the fatty acid residue and the sphingosine (or dihydrosphingosine) residue. Glycolipids have three major classes.

(a) *Cerebrosides or galactolipids*: They are ceramide-monosaccharide complexes. *Galactocerebrosides* of nerve tissue carry a ceramide made by the binding of a long-chain fatty acid to the NH_2 group of sphingosine. The CH_2OH group of the ceramide is again

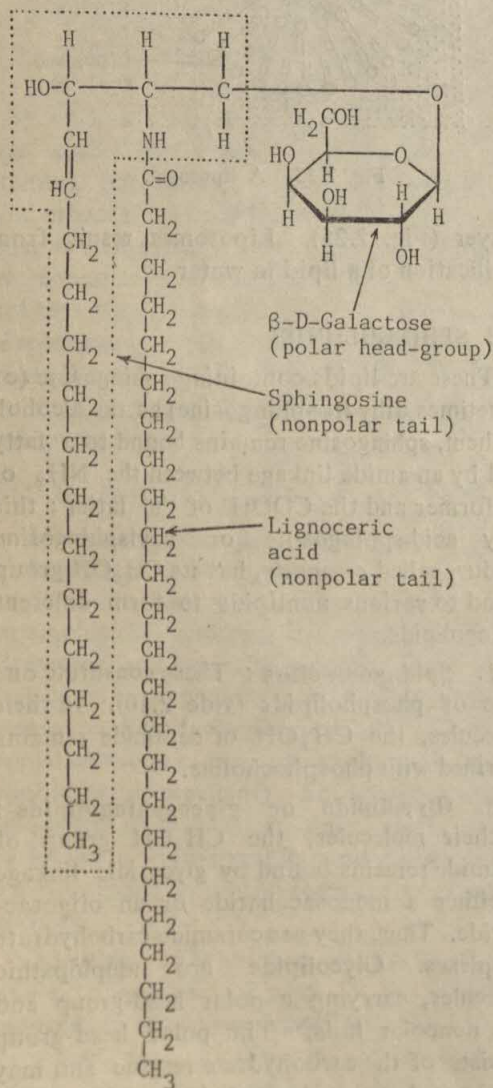


Fig. 2.26. Glycolipid molecule (a cerebroside).

connected by a β -glycosidic linkage to the C^1 of a D-galactose residue (Fig. 2.26). According to the nature of the fatty acid residue, there are four main types of cerebrosides in the nerve tissue, viz., *kerasin* carrying lignocerate (saturated C_{24} acid), *nervon* carrying unsaturated nervonate (C_{24}), *cerebron* containing cerebrionate (α -hydroxylignocerate), and *oxynervon* with oxynervonate (α -hydroxynervonate). *Glucocerebrosides*, carrying D-glucose instead of galactose, are found in spleen and non-nerve tissues. Unlike sphingomyelins, cerebrosides possess an uncharged polar head-group consisting of the sugar residue, and are neutral in reaction.

(b) *Sulfatides*: Sulfatides are cerebroside sulfates. Some of the hydroxyl groups in the galactose residue of a cerebroside are sulfated to yield a *sulfolipid* or sulfatide. Owing to the sulfate groups on the galactose of their polar head-group, the latter bears negative charges and is acidic. Liver, kidney, salivary glands and white matter of brain contain sulfatides.

(c) *Gangliosides*: These are ceramide-oligosaccharide complexes. A simple ganglioside or *monosialoganglioside* is composed of a ceramide bound to an oligosaccharide containing a single sialic acid (NANA), three molecules of hexoses (glucose and galactose) and one N-acetylgalactosamine (Fig. 2.27).

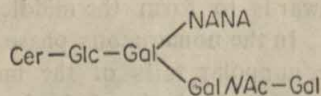


Fig. 2.27. A monosialoganglioside.

Higher gangliosides, viz., di-, tri-, tetra- and penta-sialogangliosides, carry 2, 3, 4 and 5 sialic acid residues, respectively. Gangliosides are found in spleen, RBC and nervous tissues. Due to COOH groups in the sialic acid residues of their head-groups, gangliosides are acidic in reaction and carry a negatively charged polar head-group.

2.13 STEROIDS

Steroids such as bile acids and steroid hormones are hydrocarbons possessing a fused, reduced, tetracyclic C_{17} ring system, the *cyclopentanoperhydrophenanthrene* nucleus. The first three rings (A, B and C) of the nucleus are six-membered and saturated; the last one (D) is five-membered (Fig. 2.28). Sidechains and groups are attached to different positions. In the *normal* configuration of a steroid, its A and B rings are in *cis* configuration to each other and the C^5 -hydrogen is on the same side of the plane of rings as the C^{10} -methyl group. An *allo-steroid* has its A and B rings in *trans* position to each other and the C^5 hydrogen is on the opposite side of the plane of rings compared to the C^{10} -methyl group. A substituent group lying above the plane of the rings, is called a β -oriented group, shown by a solid bond. A substituent

group lying below the plane of the rings, is an α -oriented group, the connecting bond indicated by a broken line.

Sterols are steroids with a C^3 -OH and a C_8 - C_{10} carbon chain on C^{17} , but without carboxyl and carbonyl substituents. Cholesterol, lanosterol and coprostanol are important animal sterols.

2.14 CHOLESTEROL

Cholesterol is a white crystalline solid (m.p. 149 - 150°C). It occurs in eggs, liver, brain, nervous tissue, fish-liver oils, adrenals, kidneys, blood, etc., and is known as 3β -hydroxy- Δ^5 -cholestene (Fig. 2.28). By virtue of the C^3 -OH group, cholesterol can form esters with fatty acids. It carries a C_8 -substituent on C^{17} and methyl groups on C^{10} and C^{13} .

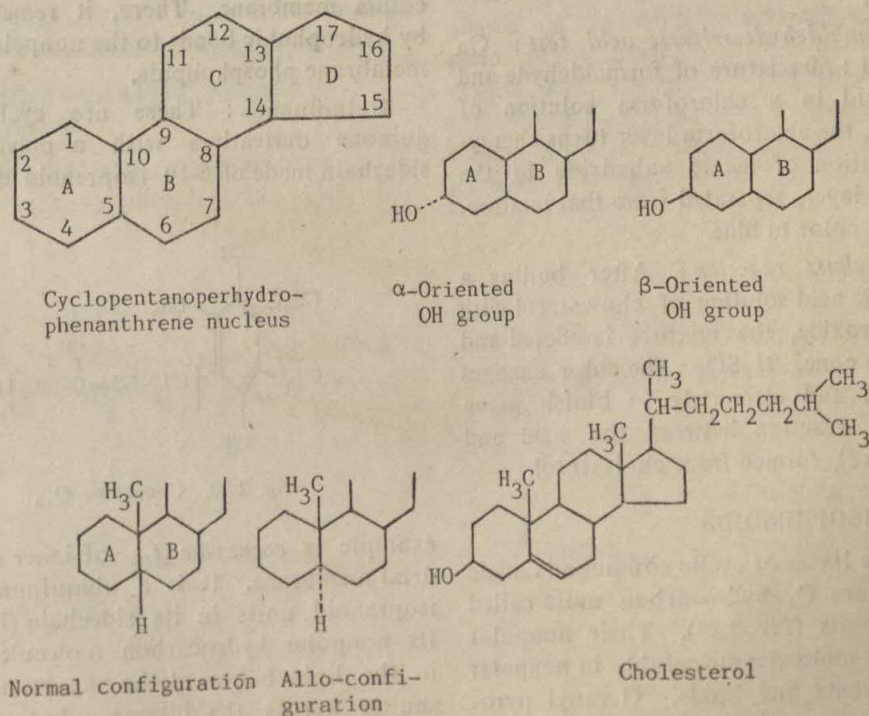


Fig. 2.28. Steroid nucleus and cholesterol.

It dissolves in ether, petroleum ether, chloroform, benzene, acetone and hot ethanol, but not in water. It can enter into water-soluble micelles, formed by bile salts, soaps and lecithins. It cannot be saponified.

Cholesterol is levorotatory. It can be oxidized to cholic acid.

Color reactions

1. *Lieberman-Burchard reaction*: Chloroform solutions of cholesterol, when treated with conc. H_2SO_4 and acetic anhydride, develop rose red, blue and green colors due to the formation of sulfonic acids and polyunsaturated cholestadienes.

2. *Salkowski reaction*: After shaking a chloroform solution of the sterol with conc. H_2SO_4 , the mixture separates on standing into a cherry-red or purple layer of chloroform and an acid layer with green fluorescence. Treatment with water decolorizes the chloroform layer.

3. *Formaldehyde-sulfuric acid test*: On adding an 1 : 50 mixture of formaldehyde and sulfuric acid to a chloroform solution of cholesterol, the chloroform layer turns cherry-red. Addition of acetic anhydride to the chloroform layer, separated from that mixture, changes the color to blue.

4. *Lipschutz reaction*: After boiling a glacial acetic acid solution of cholesterol with benzoyl peroxide, the mixture is cooled and treated with conc. H_2SO_4 ; the color changes first to red and ultimately to bluish green due to the reaction between the acid and oxysterol, formed from cholesterol.

intermediates in sterol biosynthesis by the condensation of 2, 3 and 6 isoprenoid units respectively. Other biologically important polyisoprenoids include rubber, dolichol, ubiquinones, vitamins A, E and K, and carotenes.

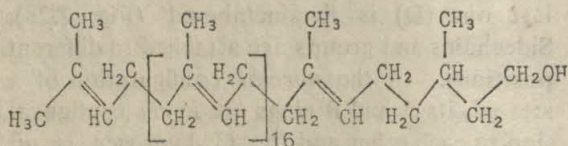


Fig. 2.29. Dolichol.

Dolichol: It is a long-chain, linear, polyisoprenyl alcohol (Fig. 2.29). Its hydrocarbon molecular chain (C_{95}) is made of 19 isoprenoid units and is as long as 10 nm when fully extended. The nonpolar hydrophobic molecule occurs in the nonaqueous middle core of the lipid bilayer of endoplasmic reticulum membrane. There, it remains bound by hydrophobic bonds to the nonpolar tails of membrane phospholipids.

Ubiquinones: These are cyclic benzoquinone derivatives with a polyisoprenoid sidechain made of 6-10 isoprenoid units. An

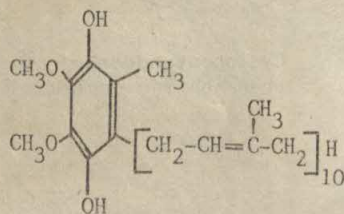


Fig. 2.30. Coenzyme Q_{10} .

2.15 POLYISOPRENOIDS

These are linear or cyclic compounds made of two or more C_5 hydrocarbon units called isoprenoid units (Fig. 2.29). Their nonpolar hydrophobic molecules are soluble in nonpolar organic solvents and lipids. Geranyl pyrophosphate, farnesyl pyrophosphate and squalene are polyisoprenoids formed as

example is *coenzyme Q_{10}* of inner mitochondrial membrane. It is a ubiquinone with 10 isoprenoid units in its sidechain (Fig. 2.30). Its nonpolar hydrocarbon molecule remains in the lipid bilayer of the membrane and can shuttle across the bilayer, but does not normally leave the latter to enter the adjoining aqueous phases.

2.16 PROSTAGLANDINS

These are long-chain, unsaturated C_{20} fatty acids having a cyclopentane (C_5) ring at the middle of the hydrocarbon chain and carrying hydroxyl and keto substituent groups (Fig. 2.31). They belong to PG_1 , PG_2 and PG_3 series, the subscript numbers denoting the number of double bonds in their linear hydrocarbon chains. In each series, there are different types like PGD, PGE, PGF, PGG and PGH, each characterized by the substituents attached to the cyclopentane ring. For example, PGD_2 , PGE_2 and PGF_2 belong to the PG_2 series, each having two double bonds in the linear sidechains (Fig. 2.31). But the substituents at C^9 and C^{11} of the ring are respectively an OH and a keto in PGD_2 and a keto and an OH in PGE_2 , while PGF_2 has OH groups at both positions. Prostaglandins occur widely in animal tissues and body fluids such as prostate, seminal vesicles, renal medulla, thymus, blood plasma and seminal fluid. They have diverse biological actions.

2.17 CHROMATOGRAPHIC SEPARATION OF LIPIDS

Partition chromatography separates various lipids such as phospholipids, fats, free

fatty acids, cholesterol and cholesteryl esters from a mixture. The separation is caused by the differential distribution of the solutes between two fluid phases. These fluid phases consist of either two immiscible liquid solvents (as in thin-layer chromatography) or a non-volatile liquid solvent and an inert gas (as in gas-liquid chromatography). One of the fluids forms the *stationary liquid phase* immobilized on a solid support like silica gel or alumina. The other constitutes the *mobile liquid or gaseous phase* flowing through that solid support. The solutes are carried by the mobile phase to different distances and are thereby separated from each other. This separation of the solutes depends on their respective *chromatographic mobilities* (R_f). R_f of a solute is the ratio of the distances migrated by it and by the moving front of the mobile phase. R_f is in turn directly proportional to the *partition coefficient* (K) of the solute between the two immiscible fluid phases. K is directly proportional to the ratio of solubilities of the solute in the mobile and stationary fluid phases. For very dilute solutions of a sparingly soluble solute, $K = s_1/s_2$, where s_1 and s_2 are the saturation solubilities of the solute at the

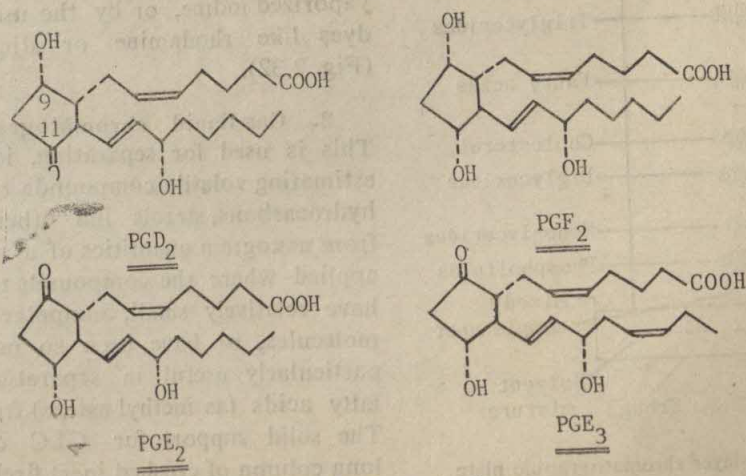


Fig. 2.31. Prostaglandins.

given temperature in the mobile and stationary fluid phases, respectively. The higher the partition coefficient of the solute, the lower is its solubility in the stationary liquid phase compared to that in the mobile phase. So, its mobility is less depressed by its dissolution in the stationary phase and it migrates over a longer distance in the direction of movement of the mobile phase. Solutes, differing in partition coefficient, thus get separated by the flowing mobile phase.

1. Thin-layer chromatography (TLC): This is a very rapid method for the separation, purification and identification of different classes of lipids and sterols. It is also used for the separation and identification of amino acids, nucleotides, sugars and drugs (for dope test of athletes). A thin layer of an inert adsorbent like silica gel, Sephadex, cellulose, alumina (for steroids) and diatomaceous earth (for sugars), is used as the solid support for TLC. The finely powdered adsorbent is thoroughly dispersed in a small volume of water by vigorous stirring to form a slurry. The latter is spread thinly and evenly on a

glass or plastic plate. It is then dried and activated by heating in an oven at 105-120°C for 15 mins or more, and cooled to room temperature. A drop of the mixed sample, dissolved in a nonpolar solvent, is placed as a spot on the adsorbent layer near one end of the plate. After drying the spot, that end of the plate is dipped vertically in a trough of a specific mixture of nonpolar solvents such as benzene-acetone or hexane-ether-formate mixture. The assembly is then kept inside a chromatographic chamber. The solvent rapidly ascends from the trough along the thin adsorbent layer due to the capillary action of the finely powdered adsorbent. While ascending along the adsorbent layer, the mobile solvent carries different solutes of the sample to different spots along the layer; the distances migrated by the solutes depend on their partition coefficients between the mobile liquid phase and the stationary liquid phase immobilized on the adsorbent particles. The mobile solvent front reaches in about $\frac{1}{2}$ hour near the upper end of the plate. The latter is removed from the chamber and dried. Finally, the separated spots are located on the plate either by first spraying it with a chromic sulfuric acid solution and then heating the plate (charring), or by exposure to vaporized iodine, or by the use of fluorescent dyes like rhodamine or dichlorofluorescein (Fig. 2.32).

2. Gas-liquid chromatography (GLC): This is used for separating, identifying and estimating volatile compounds of fatty acids, hydrocarbons, sterols and other lipids, even from nanogram quantities of a sample. It is applied where the compounds to be separated have relatively small, nonpolar and volatile molecules, or have been so modified. It is particularly useful in separating individual fatty acids (as methyl esters) from a mixture. The solid support for GLC consists of a long column of crushed inert firebricks, micro-glass beads or diatomaceous earth particles.

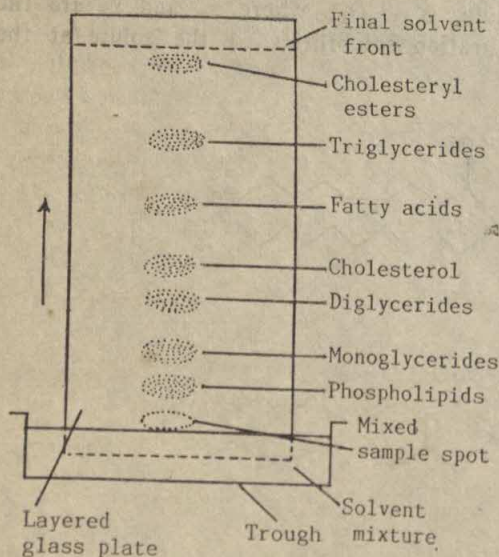


Fig. 2.32. Thin-layer chromatographic plate showing separated lipid spots.

These particles are coated with a nonvolatile liquid like silicone oil, grease or a high-boiling polyester. This liquid, immobilized on the particles, serves as the stationary liquid phase. The vaporized mixture of volatile compounds, such as methyl esters of fatty acids, is carried by a uniform flow of an inert gas through a long spiral glass or metal tube packed with a column of the coated particles (Fig. 2.33). The tube containing the column of coated particles is kept in an oven maintained at 170-225°C. The inert gas flowing through the column serves as the mobile gaseous phase. The volatile components of the mixture get separated from each other according to their partition coefficients between the mobile gaseous phase and the stationary liquid phase. The components, having higher partition coefficients, are relatively less soluble in the stationary phase and consequently move more rapidly with the mobile gaseous phase through the column to reach the distal end of the tube earlier than the components with lower partition coefficients. Thus, the individual components (e.g., fatty acid esters) emerge separately from the distal end of the tube. They are detected and identified by a detector such as

the thermal conductivity detector, the hot wire detector or the ionization detector, which records the information as a series of peaks in a chromatogram. Individual components are identified by comparing this chromatogram with that of a standard mixture of

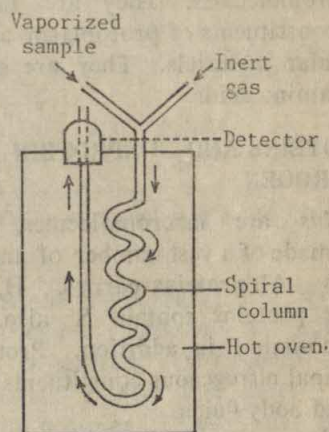


Fig. 2.33. Sketch diagram of gas-liquid chromatography apparatus.

known composition while their concentrations in the sample are obtained from the areas under the respective peaks.

3. CHEMISTRY OF PROTEINS

Proteins form the fundamental structural components of the body. They are nitrogenous macromolecules. They are the major organic constituents of protoplasm and many extracellular materials. They are composed of many amino acids.

3.1 PROTEINS AND NONPROTEIN NITROGEN

Proteins are macromolecules, each a polymer made of a vast number of amino acid molecules. All proteins carry C, H, O and N. Most proteins contain S also. Some proteins contain P in addition. Proteins are the principal nitrogenous constituents of food, tissues and body fluids.

Peptides are also molecules, composed of amino acids linked with each other. But they possess much smaller molecules than proteins and contain much fewer amino acids compared to proteins. *Dipeptides, tripeptides, oligopeptides and polypeptides* are made of respectively 2, 3, several and many amino acid molecules.

Amino acids are small biomolecules. They are organic acids in which a hydrogen attached to the carbon skeleton has been substituted by an amino (NH_2) group. An α -amino acid (e.g., alanine, valine, methionine) carries the amino group on its α -carbon, located next to the carboxyl-C (Fig. 3.1). A β -amino acid and a γ -amino acid carry the NH_2 group on the β and γ carbons respectively (e.g., β -alanine and γ -aminobutyrate). Amino acids in protein molecules are mostly α -amino acids. The α -carbon of an α -amino acid has its four valencies satisfied by the following four groups: an $\alpha\text{-NH}_2$ group, an $\alpha\text{-COOH}$ group, a hydrogen, and a fourth group called the *sidechain* or *R group*. The sidechain varies from amino acid to amino acid and includes all other carbons of the carbon skeleton except the $\alpha\text{-C}$ and the $\alpha\text{-carboxyl-C}$ (Fig. 3.1); e.g., glycine, alanine, methionine and valine possess —H , —CH_3 , $\text{—CH}_2\text{CH}_2\text{SCH}_3$ and $\text{—CH(CH}_3)_2$ as their respective sidechains. Depending on the pH of the medium, groups such as NH_2 and COOH of an amino acid

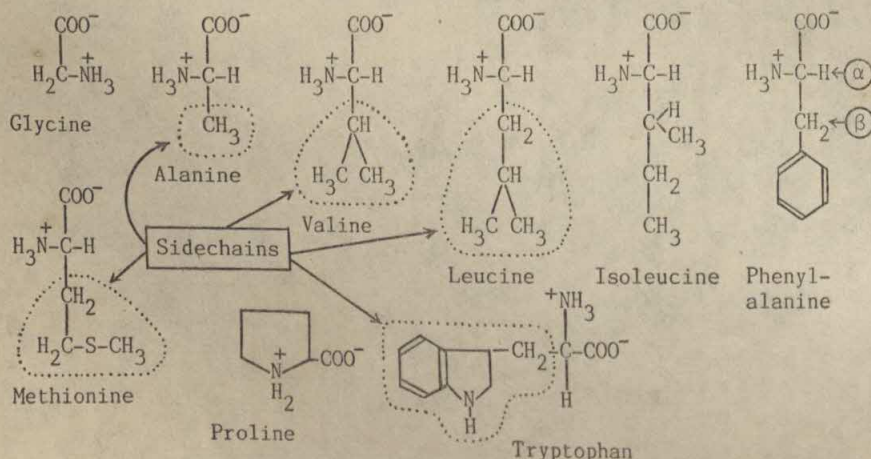


Fig. 3.1. L-Amino acids carrying nonpolar sidechains.

may remain ionized respectively as NH_3^+ , COO^- , etc.

A *peptide bond* is a covalent bond binding the carboxyl-C of one amino acid and the amino-N of another (Fig. 3.2). In protein and peptide molecules, the $\alpha\text{-COOH}$ of each amino acid is linked to the $\alpha\text{-NH}_2$ of the next amino acid by a peptide bond. A linear chain of amino acids is thus formed in the molecule and the sidechains of the amino acids project on one or other side of this peptide chain.

Nonprotein nitrogen (NPN) is the nitrogen present in nitrogenous substances other than proteins. NPN substances include free amino acids of body fluids, purines and pyrimidines occurring either free or in nucleotides and nucleic acids, creatine occurring mainly as creatine phosphate in muscles, biologically active amines like catecholamines and histamine, and nitrogenous catabolites like ammonia, urea, uric acid and creatinine. Urinary nitrogen normally consists solely of NPN. But plasma nitrogen consists mainly of protein nitrogen.

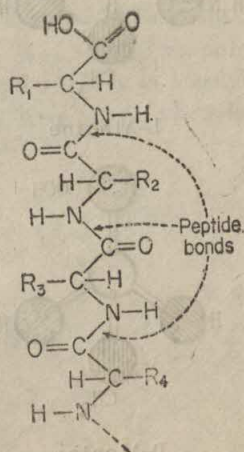


Fig. 3.2. Peptide bonds in a peptide chain.
 R_1 , R_2 , etc., are amino acid sidechains.

TABLE 3.1. *NPN in normal human blood and urine.*

<i>NPN substances</i>	<i>In blood</i> (mg dl ⁻¹)	<i>In urine</i> (g per 24 hrs)
Total NPN	20—35	20—35
Amino acids	31—47	0.1—0.2
Ammonia	0.04—0.07	0.5—1.0
Creatine	0.2—0.6*	0.06—0.15
Creatinine	0.7—1.2*	0.8—1.8
Hippuric acid	—	0.5—0.9
Urea	17—30	15—32
Uric acid	3—6*	0.2—0.8

*Values in mg per dl (100 ml) of serum.

Functions of proteins: (a) Proteins are major *structural components* of cellular membranes, organelles, cytoskeletal structures, extracellular matrix and fibres. (b) Some structural proteins like actin and myosin are *contractile proteins*; they cause movements of muscle fibres, microvilli, etc., and also rufflings of plasma membrane. (c) Some of the proteins, located in membranes or cytoplasm, function as *molecular receptors*; they bind with specific molecules reaching or entering the cell so as to mediate in the cellular actions of those molecules. (d) Some proteins like histones and protamines combine with nucleic acids to form *nucleoproteins* of chromosomes and ribosomes. (e) *Carrier proteins* occur in membranes and body fluids for binding with specific substances and transporting them either across the membranes or in the body fluids; e.g., sodium pump for transmembrane Na^+ transport, and transferrin for transport of Fe^{3+} in plasma. (f) Specific proteins may bind with and *store* specific substances in the cell or extracellular fluid, e.g., thyroglobulin stores thyroxine in thyroid vesicles. (g) All *enzymes* and the *peptide hormones* (e.g., insulin and growth hormone) are proteins. (h) Some proteins like immunoglobulins and complements of

plasma help in *body immunity*. (i) Some proteins are constituents of *respiratory pigments* like hemoglobin, myoglobin and cytochromes. (j) When necessary, proteins may also be catabolized to *produce energy*. (k) Proteins influence and control *ionic distributions* as well as the *osmotic distribution* of water on two sides of membranes.

Functions of amino acids: (a) Amino acids are joined with each other by peptide bonds to form *proteins and peptides*. (b) Some of the amino acids like glycine and alanine are converted to *carbohydrates* in the body. (c) Specific amino acids can give rise to *specialized biological products* in the body; e.g., tyrosine may give rise to the hormones *epinephrine* and *thyroxine* and to the skin pigment *melanin*; tryptophan may produce the vitamin *nicotinamide*; glycine, arginine and methionine may give rise to *creatine*; glycine and cysteine may be used in synthesizing *bile salts*; histidine may be changed to *histamine*; glycine is used in synthesizing *heme*; *pyrimidines* are synthesized from aspartate and glutamine; glycine, aspartate, glutamine and serine are used in *purine synthesis*; glutamate, cysteine and glycine are serially linked by peptide bonds to form *glutathione*, an important reducing agent of the body. (d) Some amino acids like glycine, cysteine and methionine may be used for *detoxication* of various toxic substances. (e) The methyl group in the sidechain of methionine may be transferred to various substances by a process called *transmethylation*.

3.2 ISOMERISM OF AMINO ACIDS

Amino acids show two principal types of isomerism, both depending on the presence of asymmetric carbons in the molecule. Glycine possesses no asymmetric C and consequently lack both types of isomerism.

1. Stereoisomerism: Amino acids except glycine exist in D and L isomer forms. A D-amino acid has its α -NH₂ and H oriented

around its asymmetric α -C in the same way as the OH and H, respectively, around the asymmetric middle C of D-glyceraldehyde. The corresponding L-amino acid bears the NH₂ and the H on its α -C in the same orientation as the OH and the H, respectively, of the middle C of L-glyceraldehyde (Fig. 3.3). Natural proteins of animals and plants generally contain L-amino acids. Abbreviations like Ala, Val and Ser are used for names of L-amino acids occurring in proteins. D-amino acids occur in bacteria.

2. Optical isomerism: All amino acids except glycine carry an asymmetric α -C. A few amino acids like isoleucine and threonine also carry additional asymmetric carbons in their sidechains; e.g., β -C of isoleucine and threonine. Consequently, amino acids other than glycine exhibit optical activities and exist as dextrorotatory (d) and levorotatory (l) isomers. For example, D-alanine is levorotatory (l or -) while L-alanine is dextrorotatory

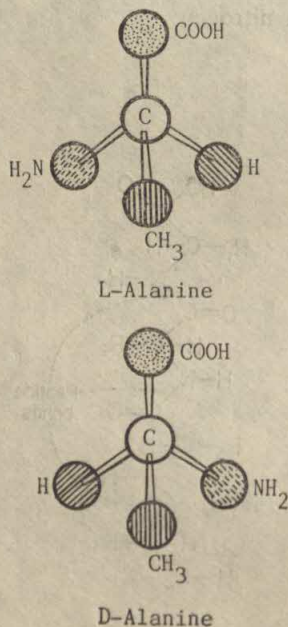


Fig. 3.3. Models of D and L isomers of alanine.

(*d* or +). Optical activity depends on pH and sidechain. Heating in acidic or alkaline pH may change an optical isomer into a racemic mixture of two antagonistic optical isomers of the amino acid.

3.3 CLASSIFICATION OF AMINO ACIDS

1. Amino acids in proteins

Animal proteins generally contain 20 α -amino acids, including one imino acid. They are usually classified according to the nature of their sidechains or R groups (Table 3.2).

1. *Amino acids with nonpolar sidechains*: They carry nonionized, nonpolar, hydrophobic (water-repellant), hydrocarbon sidechains. They are mostly *monoaminomonocarboxylic acids* having a single NH_2 and a single COOH group on the α -carbon. The nonpolar sidechains of two such amino acids may form hydrophobic bonds with one another. Nonpolar amino acids are further classified as follows:

(a) *Amino acids with nonpolar aliphatic sidechains*: They carry a branched or unbranched, open, hydrocarbon sidechain like $-\text{CH}_3$, $-\text{CH}(\text{CH}_3)_2$, $-\text{CH}_2\text{CH}(\text{CH}_3)_2$ and $-\text{CH}_2\text{CH}_2\text{S}(\text{CH}_3)_2$; e.g., alanine, valine, leucine, isoleucine and methionine (Fig. 3.1).

(b) *Amino acids with nonpolar aromatic sidechains*: They carry a phenyl or indole ring in the sidechain; e.g., phenylalanine and tryptophan (Fig. 3.1).

(c) *Imino acids*: Proline (Fig. 3.1), present in collagens, immunoglobulins and prolamines, is a heterocyclic imino acid.

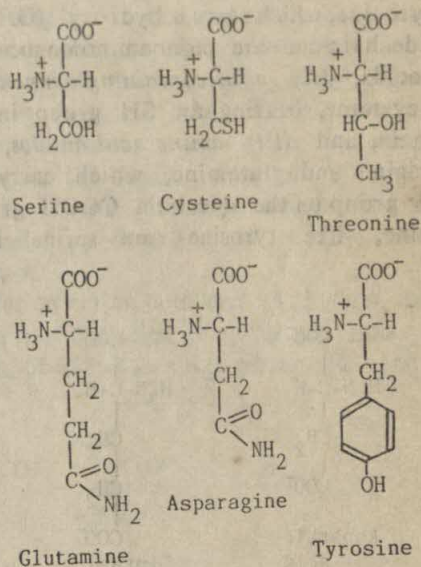


Fig. 3.4. L-Amino acids carrying uncharged polar sidechains.

2. *Amino acids with polar sidechains*: They carry sidechains bearing ionized or nonionized, hydrophilic (water-attracting) polar groups. Polar amino acids are further classified as follows:

(a) *Amino acids with uncharged polar sidechains*: They bear nonionizable polar sidechains like $-\text{CH}_2(\text{OH})$, $-\text{CH}(\text{OH})\text{CH}_3$ and $-\text{CH}_2\text{CO}(\text{NH}_2)$ (Fig. 3.4). Such sidechains

TABLE 3.2. Amino acids occurring in proteins.

Charged polar	Uncharged polar		Nonpolar	
Aspartic acid (Asp)	Serine (Ser)	Tyrosine (Tyr)	Glycine (Gly)	Valine (Val)
Glutamic acid (Glu)	Threonine (Thr)	Asparagine (Asn)	Alanine (Ala)	Leucine (Leu)
Lysine (Lys)	Cysteine (Cys)	Glutamine (Gln)	Isoleucine (Ile)	Proline* (Pro)
Arginine (Arg)			Methionine (Met)	
Histidine (His)			Phenylalanine (Phe)	
			Tryptophan (Trp)	

* Imino acid.

may form hydrogen bonds with water molecules or with the polar sidechains of other amino acids. The major subclasses are : (i) *Hydroxy amino acids*, viz., serine, threonine and tyrosine, which carry a hydroxyl (OH) in the sidechain and are monoaminomonocarboxylic acids, (ii) *sulfur-containing amino acid*, viz., cysteine, bearing an SH group in the sidechain, and (iii) *amino acid amides*, viz., asparagine and glutamine, which carry an amide group on the sidechain COOH group. Cysteine, like tyrosine and serine, is a

monoaminomonocarboxylic acid; but its SH group may bind to the SH group of another cysteine to form a *diaminodicarboxylic acid*, called cystine.

(b) *Amino acids with charged polar sidechains*: They carry ionizable polar groups like NH_2 , COOH , guanidinium, or imidazole group in the sidechain (Fig. 3.5). These ionizable groups bear charges and can form ionic or electrostatic bonds with counterion groups of other amino acids in a protein molecule. Charged polar amino acids have two major subclasses : (i) *Amino acids with positively charged sidechains* bear a cationic group like NH_3^+ in the sidechain; they include histidine with an imidazole sidechain, arginine with a guanidinium sidechain, and lysine which is a *diaminomonocarboxylic acid* having an NH_3^+ in its sidechain. (ii) *Amino acids with negatively charged sidechains* bear an anionic group like COO^- in the sidechain; they include glutamic and aspartic acids which are *monoaminodicarboxylic acids* having a COO^- group in the sidechain.

Monoaminomonocarboxylic acids, diaminodicarboxylic acids and the amino acid amides (asparagine and glutamine) are called *neutral amino acids*. They form neutral solutions because of identical numbers of acidic COOH and basic NH_2 groups in their molecules. Monoaminodicarboxylic acids and diaminomonocarboxylic acids are *acidic* and *basic amino acids* respectively, as they carry an excess of respectively COOH and NH_2 groups.

2. Amino acids not occurring in proteins

They occur either in free forms or in specific complexes. They include β -alanine (in coenzyme A, ACP and pantothenate), γ -aminobutyrate (a neurotransmitter in brain), iodothyronines (the thyroid hormones T_4 and T_3), taurine (in the bile salt taurocholate), ornithine and citrulline (urea cycle

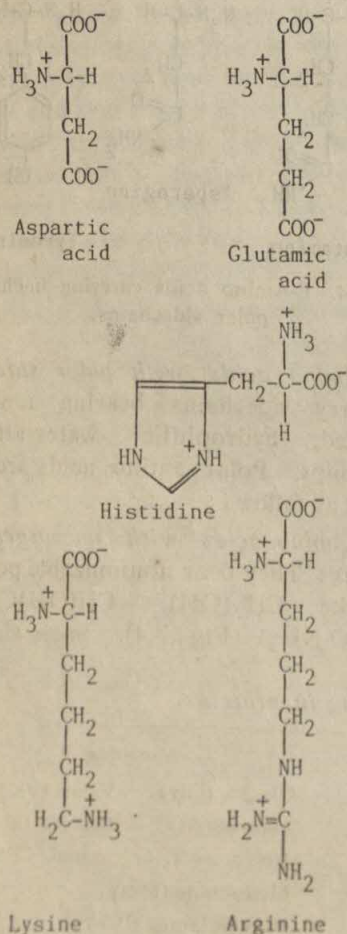


Fig. 3.5. L-Amino acids carrying charged polar sidechains,

intermediates), dopa (melanin precursor) and 3-hydroxyproline (Fig. 3.6).

3.4 PROPERTIES OF AMINO ACIDS

Amino acids are colorless, crystalline, water-soluble solids with high melting points.

1. **Amphoteric nature:** Depending on the pH, the α -NH₂ and the sidechain NH₂, imidazole and guanido groups of amino acids may accept protons (H⁺) to form cations while the α -COOH and the sidechain COOH groups may donate H⁺ to form anions. An amino acid consequently behaves as an *ampholyte*, acting both as a donor and as an acceptor of H⁺. At a specific pH called the *isoelectric*

pH (pI), the amino acid exists as a *dipolar ion* or *zwitterion* carrying equal numbers of positive and negative charges on its ionizable groups so that the net charge is minimum or zero (Fig. 3.7). Consequently, the zwitterion moves neither to the cathode nor to the anode in an electric field. If the pH of the solution is lower than the pI, the amino acid molecule exists as a cation by accepting H⁺ from the acidic solution. If the pH exceeds the pI, the same amino acid forms an anion by donating H⁺ to the alkaline solution.

For an amino acid such as glycine, having either a nonpolar or an uncharged polar side-chain, $pI = \frac{1}{2}(pK_a + pK_b)$ where pK_a and pK_b

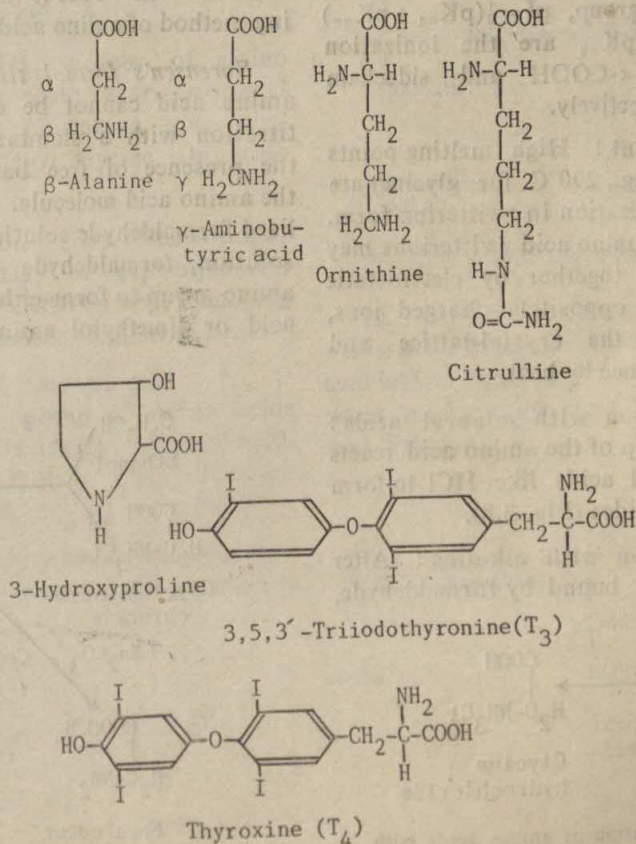


Fig. 3.6. Amino acids not occurring in proteins.

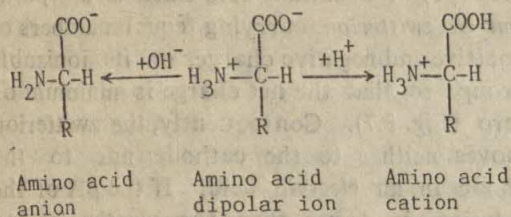


Fig. 3.7. Amino acid ions at different H^+ concentrations.

are the ionization exponents of its α -COOH and α -NH₂ groups respectively. For a diamino acid such as lysine with a sidechain NH_3^+ group, $\text{pI} = \frac{1}{2}(\text{pK}_{b1} + \text{pK}_{b2})$ where pK_{b1} and pK_{b2} are the ionization exponents of its α -NH₂ and sidechain NH_2 groups respectively. For a dicarboxylic acid such as glutamate with a sidechain COO^- group, $\text{pI} = \frac{1}{2}(\text{pK}_{a1} + \text{pK}_{a2})$ where pK_{a1} and pK_{a2} are the ionization exponents of its α -COOH and sidechain COOH groups respectively.

2. **Melting point:** High melting points of amino acids (e.g., 290°C for glycine) are due to their crystallization in zwitterion form. A large number of amino acid zwitterions may be strongly held together by electrostatic bonds between their oppositely charged ions, thereby stabilizing the crystal-lattice and resisting its disturbance by heat.

3. **Salt formation with mineral acids:** The basic NH_2 group of the amino acid reacts with strong mineral acids like HCl to form salts like hydrochlorides (Fig. 3.8).

4. **Salt formation with alkalis:** After the amino group is bound by formaldehyde,

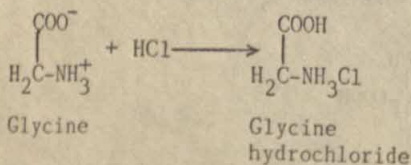


Fig. 3.8. Salt formation of amino acids with strong mineral acids.

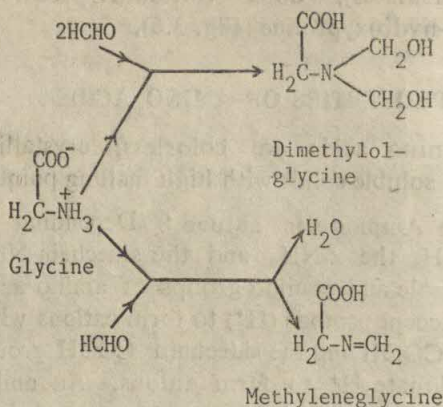


Fig. 3.9. Reaction of glycine with formaldehyde.

the carboxyl group may react with alkalis to form salts. This is the basis of the following method of amino acid estimation.

Sorensen's formal titration method: An amino acid cannot be directly estimated by titration with a standard alkali, because of the presence of free basic amino group in the amino acid molecule. On adding neutralized formaldehyde solution to an amino acid solution, formaldehyde combines with the amino group to form either methylene amino acid or dimethylol amino acid (Fig. 3.9);

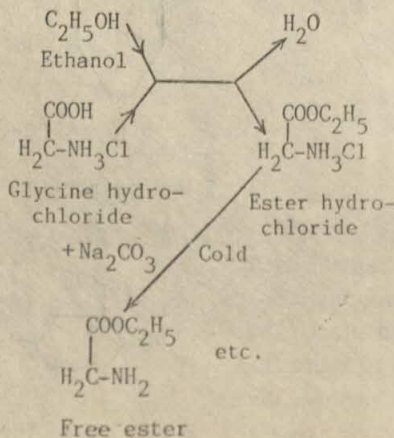


Fig. 3.10. Esterification of glycine.

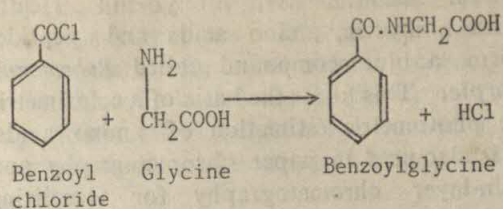


Fig. 3.11. Acylation of amino acid.

these products are strong acids and may be estimated by titration with a standard alkali.

5. Esterification: The COOH group of an amino acid may be esterified with alcohol, only after the basic NH_2 group has been bound by HCl. Treatment with Na_2CO_3 solution in cold releases the free ester from the ester hydrochloride (Fig. 3.10).

6. Acylation: NH_2 groups of amino acids may react with acyl anhydrides or acyl halides like benzoyl chloride to give acyl amino acids like benzoyl glycine (hippuric acid) (Fig. 3.11).

7. Decarboxylation: Dry distillation, or heating with Ba(OH)_2 or diphenylamine evolves CO_2 from the COOH group, changing the amino acid to an amine (Fig. 3.12).

8. Reaction with nitrous acid: N_2 is evolved from the NH_2 group of amino acids (but not from imino acids) by nitrous acid. The amino acid is thereby changed to an α -hydroxy acid (Fig. 3.13).

Van Slyke's method for amino-N estimation: In this method, the amino acid solution is

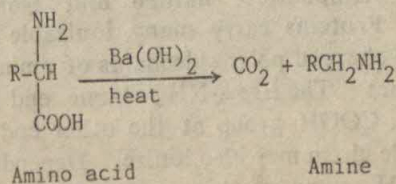


Fig. 3.12. Decarboxylation of amino acid.

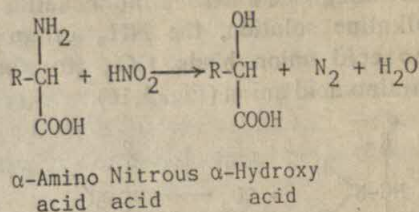


Fig. 3.13. Reaction of amino acid with nitrous acid.

treated with HNO_2 and the volume of N_2 gas evolved is measured at STP.

9. Formation of amide: Anhydrous NH_3 replaces the alcohol in an amino acid ester to produce the amino acid amide (Fig. 3.14).

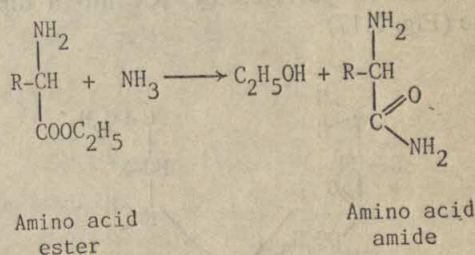


Fig. 3.14. Formation of amino acid amide.

10. Oxidation: Permanganate or H_2O_2 oxidizes the NH_2 group to change the amino acid into an imino acid. The latter reacts with water to dissociate into NH_3 and an α -keto acid (Fig. 3.15).

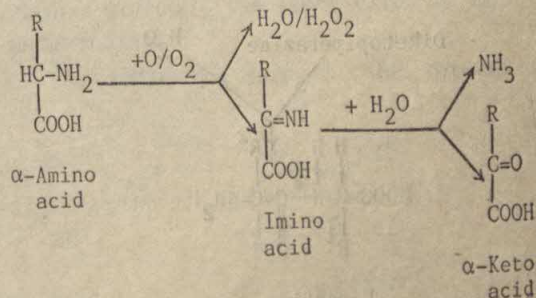


Fig. 3.15. Oxidative deamination.

11. Siegfried's carbamino reaction: In an alkaline solution, the NH_2 group of an amino acid anion binds CO_2 to form a carbamino acid anion (Fig. 3.16).

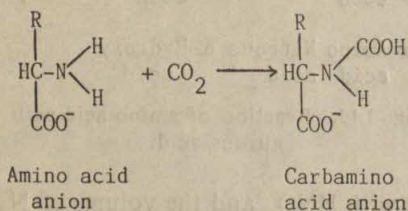


Fig. 3.16. Siegfried's carbamino reaction.

12. Peptide formation: On being heated, two amino acid esters are linked by two peptide bonds to form 2,5-diketopiperazine. The latter is hydrolyzed by HCl into a dipeptide (Fig. 3.17).

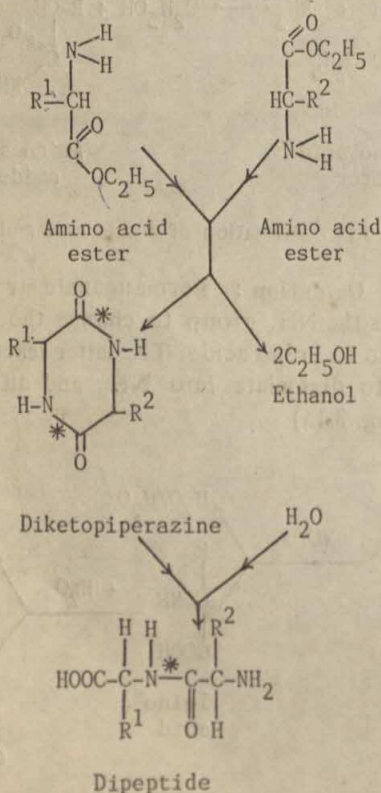


Fig. 3.17. Peptide formation. Asterisks mark peptide bonds.

13. Reaction with ninhydrin: Heated with ninhydrin, amino acids and peptides form a blue compound called *Ruhemann's purple*. This forms the basis of a colorimetric or photometric estimation of amino acids. It is also used in paper chromatography and thin-layer chromatography for visualizing amino acids separated in different spots on the chromatographic paper or on the thin layer of adsorbent powder.

14. Formation of Schiff bases: The amino acid may bind reversibly to the aldehyde group of an aromatic compound like pyridoxal phosphate to form a Schiff base (Fig. 3.18).

15. Oxidation of sulfhydryl groups: Two cysteine molecules may be joined by an S-S linkage to form cystine, on oxidation of their sidechain SH groups by mild oxidizing agents. Cystine can be reductively cleaved at its S-S bond with β -mercaptoethanol or glutathione giving two cysteine molecules.

16. Specific color reactions: For Millon, Sakaguchi, Hopkins-Cole and other color reactions, vide. 3.5.

3.5 PROPERTIES OF PROTEINS

1. Molecular weight: Proteins are macromolecules. They have high molecular weights (e.g., myoglobin - 17450, adult hemoglobin - 64500, serum albumin - 69000, γ -globulin - 156000, thyroglobulin - 670000); MW may be estimated by molecular sieve chromatography or by determining sedimentation rate, osmotic pressure, freezing point, light scattering effect, X-ray diffraction, turbidity, etc. Large molecules give the proteins colloidal properties.

2. Amphoteric nature and isoelectric pH: Proteins carry many ionizable groups in the charged polar sidechains of amino acid residues. The free $\alpha\text{-NH}_2$ at one end and a free $\alpha\text{-COOH}$ group at the other end of the peptide chain may also ionize. Depending on the pH, some of these groups act as proton donors and others act as proton acceptors.

So, proteins are *ampholytes*, acting both as donors and acceptors of H^+ and consequently carrying both anionic and cationic groups. At a specific pH called the *isoelectric pH* (pI), a protein exists as a *dipolar ion* or *zwitterion*, carrying equal number of positive and negative

exponents (pK). If the basic amino acids carrying cationic sidechains (e.g., lysine, arginine or histidine) predominate in a protein, its pI resides in the alkaline range. Such a protein forms a cation and behaves as a base at the physiological pH of 7.4, and is

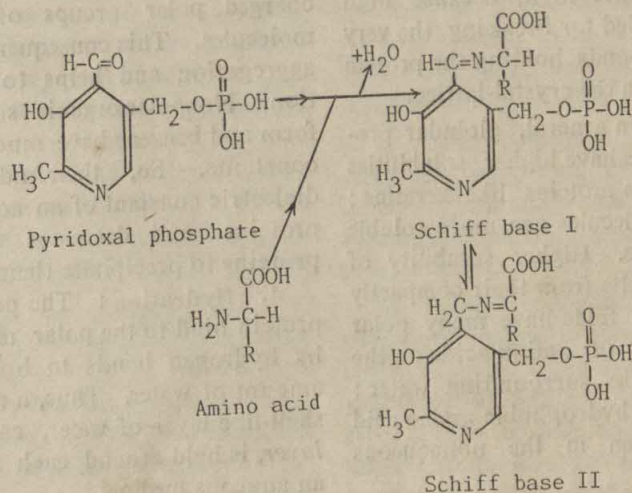


Fig. 3.18. Formation of Schiff base.

charges on its ionizable groups. So, its net charge becomes negligible or zero at its pI . Consequently, the protein zwitterion moves to neither electrode in an electric field at its pI . On the acidic side of its isoelectric pH, a protein exists as a cation by accepting H^+ from the solution (Fig. 3.19). But on the alkaline side of its pI , a protein becomes an anion by donating H^+ .

The isoelectric pH depends on relative numbers of cationic and anionic sidechains in the amino acid residues and their ionization

called a *basic protein*; e.g., histones and protamines. If the acidic amino acids carrying anionic sidechains (e.g., glutamate or aspartate) predominate in a protein, the latter has the isoelectric pH in the acidic range. Such a protein forms an anion at the physiological pH of 7.4 and is called an *acidic protein*; e.g., pepsin. As the normal pH of blood plasma is on the alkaline side of the pI s of plasma proteins, the latter exist as anions in the plasma.

The zwitterion form of the protein is

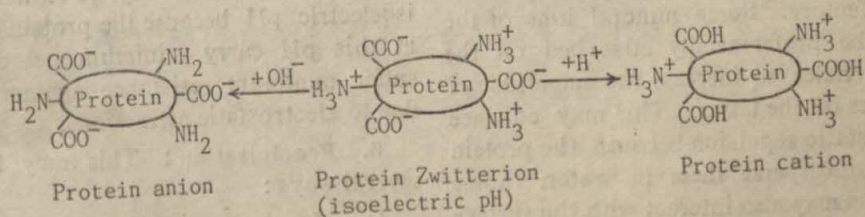


Fig. 3.19. Protein ions at different H^+ concentrations.

responsible for its high dipole moment and high dielectric constant. Proteins exist in zwitterion forms in their crystal-lattices. Numerous electrostatic bonds between the opposite charges of different zwitterions stabilize the crystal-lattice structure. *Melting points* of proteins are high, because high temperatures are needed for breaking the very many electrostatic bonds holding the protein zwitterions together in the crystal-lattice.

3. **Solubility** : In general, globular proteins such as albumins have higher solubilities than elongated fibrous proteins like keratins ; moreover, smaller molecules are more soluble than larger molecules. Higher solubility of globular proteins results from their compactly folded forms. These folds have many polar hydrophilic sidechains of amino acids on the surface exposed to the surrounding water ; most of the nonpolar hydrophobic amino acid sidechains lie hidden in the nonaqueous interior of the folds.

The protein zwitterions at the isoelectric pH have minimum net charges and consequently possess minimum electrostatic repulsive forces which normally prevent the aggregation and precipitation of protein molecules. So, at the isoelectric pH, the protein has the minimum solubility. If dilute acids or alkalis are added to such solutions, the protein forms cations or anions respectively ; all its ions now being similarly charged, electrostatic repulsive forces increase between them and consequently raise the solubility.

Many proteins such as globulins have increased solubilities in dilute neutral mineral salt solutions (*salting in*). This may result from two factors. Some mineral ions of the salt may be preferentially adsorbed on the protein particles to increase the magnitude of like charges on the latter. This may enhance the electrostatic repulsion between the protein particles to disperse them in water. Some mineral ions may also interact with the surface ionic groups of protein particles to suppress

their ionization into antagonistic ionic groups on the particle surface. This may reduce the mutual electrostatic attraction and aggregation of the particles.

Due to its high dielectric constant, water reduces the electrostatic attraction between the charged polar groups of different protein molecules. This consequently decreases their aggregation and helps to keep them in solution. Nonpolar organic solvents like chloroform and benzene have much lower dielectric constants. So, their addition lowers the dielectric constant of an aqueous solution of proteins and decreases the solubility of proteins to precipitate them.

4. **Hydration** : The polar groups of the protein bind to the polar molecules of water by hydrogen bonds to hold a considerable amount of water. Thus, a relatively immobile shell-like layer of water, called the *solvation layer*, is held around each protein particle in an aqueous medium.

5. **Viscosity** : Viscosity of a protein solution results from the considerable restriction in the free movement of water molecules in that solution. This depends on (i) the immobilization of significant amounts of the solvent in the solvation layer around the protein particles, (ii) large sizes of the protein particles, (iii) their elongated, coiled, three-dimensional forms, and (iv) ionic groups on their surface. So, higher the molecular weight, more elongated the molecular shape or higher the concentration of the protein, greater is the viscosity. pH also affects the viscosity of a protein solution by affecting the ionization of the proteins. Viscosity is lowest at the isoelectric pH because the protein zwitterions at this pH carry minimum net charges and suffer minimum resistance against their flow due to electrostatic attractions.

6. **Precipitation** : This may be done in several ways :

(a) *Isoelectric precipitation* : If the pH of a protein solution is adjusted to the pI

of that protein, its molecules form zwitterions. These carry minimum net charges and consequently exert minimum electrostatic repulsion. So, these zwitterions easily aggregate and precipitate without denaturation.

(b) *Salting out*: Concentrated solutions of neutral mineral salts such as MgSO_4 , Na_2SO_4 and $(\text{NH}_4)_2\text{SO}_4$ may precipitate a protein from its solution. Mineral ions attract water molecules and consequently remove the solvation layer from around protein particles. Mineral ions may also bind with oppositely charged ionic groups of proteins to reduce their surface charges. Both these enhance the aggregation and precipitation of protein particles.

(c) *By nonpolar organic solvents*: Non-polar organic solvents such as chloroform possess much lower dielectric constants than water. Their addition lowers the dielectric constant of an aqueous protein solution. This enhances the electrostatic attraction between the antagonistic ions of proteins and consequently facilitates their aggregation and precipitation.

(d) *By heavy positive or negative ions*: On the acidic side of its pI, a protein remains as cation and may then be precipitated by heavy anions like tungstate, trichloroacetate and picrate. To precipitate plasma proteins for estimating blood sugar (*Folin-Wu method*), $\frac{2}{3}\text{N}$ H_2SO_4 and 10% sodium tungstate are added to blood. H_2SO_4 renders the medium far more acidic than the isoelectric pHs of plasma proteins. Protein cations, formed consequently, bind with tungstate anions to get precipitated as protein tungstate.

On the alkaline side of its pI, a protein exists as anion and may then be precipitated as metal proteinates by heavy metal ions like Zn^{2+} . For example, to precipitate plasma proteins in the *Nelson-Somogyi method* for the estimation of blood glucose, $\text{Ba}(\text{OH})_2$ and ZnSO_4 solutions are mixed with the blood. $\text{Ba}(\text{OH})_2$ makes the medium more alkaline

than the isoelectric pHs of the plasma proteins; the plasma protein anions, formed consequently, bind with Zn^{2+} to get precipitated as zinc proteinates.

(e) *By specific antibodies*: Antibodies are γ -globulins released by plasma cells under the inducing action of foreign proteins called *antigens*. Each type of antibody may bind with and precipitate the specific antigenic protein which has induced its production.

7. *Color reactions*: Many color reactions are produced by the sidechains of specific amino acids and are given by those amino acids both in the free state and in the peptide chain.

(a) *Xanthoproteic reaction*: Proteins containing phenolic or indolic amino acids (phenylalanine, tyrosine and tryptophan) give yellow precipitate on being boiled with conc. HNO_3 . On adding an alkali, the precipitate turns orange due to the formation of nitro compounds involving the phenolic and indolic amino acid residues. Collagens are deficient in these amino acids. So, gelatins produced from collagens cannot give this test.

(b) *Millon's reaction*: Proteins containing the phenolic amino acid tyrosine give a white precipitate on being mixed with Millon's reagent which contains mercuric nitrate, mercurous nitrate, nitric acid and a small amount of nitrous acid. Heating produces a red phenolic complex of mercury, turning the precipitate red. Peptones give a red solution. Gelatins, deficient in tyrosine, do not give this test.

(c) *Sakaguchi reaction*: Proteins containing arginine give this reaction. On boiling the protein solution with Sakaguchi's reagent containing sodium hypochlorite and α -naphthol, a red color is produced.

(d) *Pauly reaction*: This reaction is positive for proteins containing either tyrosine or histidine. On treating such proteins with diazotized sulfanilic acid in alkaline

solution, a red color is produced by a coupling reaction.

(e) *Hopkins-Cole reaction*: A protein solution is mixed with Hopkins-Cole reagent (containing glyoxylic acid). Conc. H_2SO_4 is then added gently to that mixture to form a separate lower layer. A violet or purple ring appears at the junction of the two layers if the protein contains tryptophan.

(f) *Adamkiewicz reaction*: A protein solution is first mixed with glacial acetic acid which contains glyoxylic acid as impurity. The mixture is then layered over conc. H_2SO_4 . A purple ring appears at the junction of the two layers if the protein contains tryptophan.

(g) *Acree-Rosenheim reaction*: A protein solution is mixed with dilute formaldehyde solution. The mixture is then layered over conc. H_2SO_4 . A violet ring appears at the junction of the two layers if the protein contains tryptophan.

Gelatins are deficient in tryptophan. So, they do not respond to Hopkins-Cole, Adamkiewicz and Acree-Rosenheim tests.

(h) *Nitroprusside reaction*: If the solution of a cysteine-containing protein is treated with sodium nitroprusside in dilute NH_4OH solution, a red color develops.

(i) *Biuret reaction*: This reaction is given by compounds having two or more peptide bonds, e.g., proteins, peptides (except dipeptides) and biuret (formed by heating urea). If the protein solution is treated with very dilute CuSO_4 solution and dilute NaOH solution, a violet color (pink for peptide) is produced due to the formation of colored complexes involving the nitrogens of more than one peptide linkage and the oxygen of water. This is the basis of the *biuret test* for proteins and the *biuret formation test* for urea.

8. *Cleavage of disulfide bonds*: Disulfide bonds (S-S bonds) in a protein molecule

may be broken either by oxidation with performic acid, or by reduction to SH groups with glutathione or β -mercaptoethanol.

9. *Denaturation and coagulation*: Heat, X-rays, ultraviolet rays, high pressure, concentrated mineral acids, acetone, alcohols, urea, heavy metal ions, detergents, sodium dodecyl sulfate, violent agitation and even dilution may rupture noncovalent bonds between amino acid sidechains in the peptide chain of a native protein. This may unfold the natural coils of the protein chain to change it into a more elongated or fibrous molecule. Such a change of a protein molecule from its natural three-dimensional form to an altered form is called the denaturation of the protein. But denaturation is not accompanied by any hydrolysis of the covalent peptide bonds. So, the amino acid sequence, i.e., the primary structure of the protein is not altered by denaturation. Thus, the molecular weight and the osmotic pressure do not change much. But isoelectric pH, levorotation and viscosity may increase while solubility, hydration, crystallization, biological activities and antigenic properties may be reduced or lost. Surface tension, absorption spectrum, digestibility and many chemical properties are also changed on denaturation.

Coagulation is the flocculation and separation of protein particles from an aqueous solution. It may be caused by freezing, heating, mechanical agitation, ultrasounds, electromagnetic fields, radiations, electrolytes (particularly heavy metal ions), etc. Sufficiently high concentrations of electrolyte ions may coagulate proteins, most effectively near the isoelectric pH of the protein. Coagulating effect of an electrolyte is due to its 'salting out' action and rises with the valency and charge of the mineral ion antagonistic to the surface ions of the protein. Coagulation may result on freezing, because of the formation of pure ice crystals and the consequent increase in concentrations of protein and electrolyte in

the remaining solution. Heat coagulates a protein by breaking its noncovalent bonds and thereby uncoiling its native globular form to a denatured fibrous form. Heat coagulation varies with the pH. The rate of rise in protein denaturation due to heat is several hundred-folds that in other chemical reactions. Moreover, thermal coagulation is often irreversible.

On the contrary, many denaturations such as the urea-mediated denaturation of pancreatic ribonuclease are spontaneously reversible on removal of the denaturing agent. If the native folded form of the protein has a lower free energy than its denatured unfolded form, the latter may easily and spontaneously refold itself into the original native conformation. But if the native folded protein has a higher free energy than its denatured unfolded product, the latter cannot be *renatured* by a spontaneous folding of its peptide chain.

3.6 CLASSIFICATION OF PROTEINS

Proteins are broadly classified into simple, conjugated and derived proteins.

1. Simple proteins

These are composed of amino acids only and possess no nonprotein part in the molecule. They are usually classified further according to their solubilities and heat coagulabilities. Both these properties depend on the molecular size and shape. Globular proteins have their peptide chains compactly folded into globular forms, exposing mostly polar sidechains of their amino acid residues on the surface and hiding most of the nonpolar sidechains within the folds. This makes them soluble in polar solvents like water. Smaller molecules are more soluble than larger molecules. Elongated fibrous protein molecules have comparatively more of the nonpolar sidechains exposed on their surfaces and are consequently far less soluble. A globular protein with a large molecule, may be coagulated by heat to an insoluble fibrous form.

Major classes of simple proteins are given below.

(a) *Protamines*: These have the smallest molecules among proteins. They are soluble in water, dilute acids and alkalis, and dilute ammonia. Protamines cannot be coagulated by heat. They do not contain cysteine, cystine tyrosine and tryptophan, but have a large excess of amino acids with cationic polar sidechains, particularly arginine. Their isoelectric pHs are consequently on the alkaline side of the normal blood pH of 7.4. So, they exist as cationic or *basic proteins* in the body. These combine with nucleic acids to form nucleoproteins; e.g., salmine, sardinine and cyprinine of fish sperms and testes.

(b) *Histones*: These are also rich in cationic polar amino acids such as arginine and histidine. So, histones are also *basic proteins* with their isoelectric pHs in the alkaline range. But they have larger molecules and lower solubilities than protamines. Histones dissolve in water, dilute acids or dilute alkalis, but not in dilute ammonia. Still, they have quite small molecules and are not coagulated by heat. They form conjugated proteins with porphyrins and nucleic acids; e.g., globin of hemoglobin, and nucleohistones of chromosomal nucleoproteins.

(c) *Albumins*: These have large globular molecules and are easily thermocoagulated. They dissolve in acids, alkalis and water. They are precipitated by full saturation with $(\text{NH}_4)_2\text{SO}_4$, but not by half-saturation. Ovalbumin (egg-white) and lactalbumin (milk) are glycoproteins, but serum albumin (plasma) carries no oligosaccharide. Albumins generally have low isoelectric pHs. Consequently, they are *acidic proteins* at the pH 7.4 of body fluids and exist there as anions. They are generally deficient in glycine.

(d) *Globulins*: These have still larger and globular molecules and lower solubilities. They are insoluble in acids, alkalis and water, but dissolve in dilute neutral salt solutions.

Globulins are precipitated by half-saturation with $(\text{NH}_4)_2\text{SO}_4$ and are easily thermocoagulated. Many globulins bind with heme to form hemoproteins (e.g., hemopexin of plasma), with lipids to form lipoproteins (e.g., plasma VLDL), with metals to form metalloproteins (e.g., transferrin and ceruloplasmin of plasma), and with oligosaccharides to form glycoproteins (e.g., serum immunoglobulins).

(e) *Glutelins*: These are plant proteins, insoluble in water or neutral salt solutions, but soluble in dilute acids or alkalis; e.g., oryzenin of rice and glutenin of wheat. Having sufficiently big molecules, they are coagulated by heat.

(f) *Prolamines*: These are alcohol-soluble plant proteins. They are insoluble in water, salt solutions and absolute alcohol. But they dissolve in 50-80% ethanol, aqueous solutions of other lower alcohols, dilute acids or alkalis. They are very rich in proline, but poor in lysine; e.g., gliadin of wheat and hordein of barley.

(g) *Scleroproteins*: These are fibrous animal proteins of very low solubilities.

(i) *Keratins* are very insoluble and indigestible. α -*Keratins* occur in skin, hair, nail and wool. They are stretched reversibly by steam heating due to the reversible cleavage of intrachain bonds. They are rich in cystine which accounts for intrachain S-S linkages. The molecule remains in regular helical coils and is poor in proline and hydroxyproline. β -*Keratins* of silk, reptilian scales and spider's web contain large proportions of small and uncharged amino acids like glycine and alanine; but they are deficient in cystine, proline and hydroxyproline. They are not stretched by steam heating. The molecules remain closely packed in the form of extended pleated sheets. (ii) *Collagens* occur in cartilage, bone, tendon, and white fibre bundles of areolar tissue. These dissolve in strong acids. Collagens are changed to soluble gelatins by

boiling in water. They are rich in glycine, alanine, 4-hydroxyproline and proline, but are deficient in tryptophan, tyrosine, cysteine, cystine and methionine. Individual collagen chains are tightly hydrogen-bonded to form triple-stranded helices. Collagens are digested by pancreatic collagenase. (iii) *Elastins* are present in yellow elastic fibres of areolar tissue, yellow elastic tissue, ligaments and tendons. These are not gelatinized on boiling with water. They contain considerable amounts (>90%) of nonpolar amino acids such as alanine, leucine, valine and proline. But they do not contain cysteine, cystine, methionine, 5-hydroxylysine and histidine. Elastins are hydrolyzed by pancreatic elastase.

2. Conjugated proteins

These are complexes of simple proteins with nonproteins. The protein part of the molecule is called the *apoprotein*, the non-protein part is called the *prosthetic group*, and the entire molecule is known as a *holoprotein*. Conjugated proteins are classified according to the prosthetic group.

(a) *Chromoproteins*: Their prosthetic groups are pigments like porphyrins, carotenoids and flavins; e.g., *hemoproteins* such as cytochromes and hemoglobin contain iron-porphyrin complexes, and *flavoproteins* like Warburg's yellow enzyme contain riboflavin derivatives.

(b) *Metalloproteins*: These are metal-protein complexes; e.g., carbonic anhydrase of RBC contains Zn^{2+} , cytochrome oxidase of mitochondria contains Cu^{2+} and Fe^{3+} , ferritin of intestinal mucosa possesses Fe^{3+} , and ceruloplasmin of plasma contains Cu^{2+} and Cu^+ .

(c) *Nucleoproteins*: *Deoxyribonucleoproteins* occur in nuclear chromosomes, chloroplast stroma and mitochondrial matrix. They contain DNA as the prosthetic group. The apoprotein consists mainly of basic proteins, particularly histones, and smaller amounts of

nonhistone acidic proteins. *Ribonucleoproteins* occur in nucleoli and also form ribosome granules. They have RNA as the prosthetic group.

(d) *Phosphoprotein*: Their prosthetic groups contain phosphates, but not as phosphate-containing substances such as nucleic acids and phospholipids; e.g., caseins (milk) and ovovitellin (egg yolk). Often the phosphate remains esterified with the β -OH of an amino acid of the apoprotein.

(e) *Lipoproteins*: Their prosthetic groups are formed of triglycerides, phospholipids, sphingolipids, fatty acids and cholesterol. The nonpolar sidechains of amino acid residues of the *apolipoprotein* are bound by hydrophobic bonds with the nonpolar portions of lipids. They are abundant in cellular membranes, plasma, milk, egg yolk, etc. Plasma lipoproteins are classified according to their densities. The density varies inversely with the percentage of lipids in the molecule.

(f) *Mucoproteins and glycoproteins*: Oligosaccharide prosthetic groups constitute from less than 1 to more than 80 percent of these molecules. Oligosaccharide chains are bound either by glycosidic linkages with the sidechain OH of some hydroxy amino acid residues of the apoprotein, or by glycosylamine linkages with the sidechain amide-N of asparagine residues of the apoprotein. Two major classes are glycoproteins and proteoglycans. *Glycoproteins* carry galactose, mannose, N-acetylhexosamines, fucose, xylose, arabinose and sialic acids, but no hexuronic acid, in their oligosaccharide chains. Glycoproteins include mucins, immunoglobulins, complements, many enzymes, some hormones like TSH, FSH and LH, and many structural proteins like collagens. In *proteoglycans*, the polysaccharide is a mucopolysaccharide such as chondroitin sulfate, dermatan sulfate, heparan sulfate and keratan sulfate. Oligosaccharides of proteoglycans are made of mainly hexuronic acids and N-acetylated

hexosamines (both often O-sulfated); sialic acids, xylose, galactose and N-sulfated glucosamines occur in much smaller amounts. Proteoglycans form structural components.

3. Derived proteins

These are produced from natural (*native*) proteins by various physical and chemical factors.

(a) *Denatured or primary derived proteins*: Water, acids, alkalies, heat, radiations, etc., may change native proteins nonhydrolytically into denatured proteins. They possess molecular weights similar to the original native proteins, but differ from them in solubility, precipitation and crystallization. For example, heat, X-rays, ultraviolet rays, agitation, alcohol or urea change soluble globular proteins like ovalbumin to fibrous, insoluble, *coagulated proteins*. The latter are difficult to crystallize. Water denatures globulins into *proteans*. Gastric HCl and intestinal bicarbonates convert native proteins into *acid* and *alkali metaproteins*, respectively. These are insoluble in water or neutral salt solutions. Metaproteins and proteans are precipitated by half-saturation with ammonium sulfate.

(b) *Secondary derived proteins*: Progressive hydrolysis of peptide bonds breaks the protein into progressively smaller molecules, viz., *proteoses*, *peptones* and *peptides*. They are generally soluble in water and not coagulated by heat. Most proteoses are precipitated by full saturation with $(\text{NH}_4)_2\text{SO}_4$. But the latter cannot precipitate peptones or peptides.

3.7 STRUCTURE OF PROTEINS

Each molecule of protein is composed of many molecules of amino acids, joined by peptide bonds. The molecular structure has four orders.

I. Primary structure

The primary structure of a protein molecule is the sequence of amino acids in its peptide

chain. The primary structure is formed by *covalent peptide bonds* linking the α -carboxyl carbon of each amino acid with the α -amino nitrogen of the next amino acid (Fig. 3.20). The carbon and nitrogen involved in a peptide bond and the α -carbons of the two amino acids, connected by that bond, lie in the same plane. The backbone of the peptide chain consists of the α -C, α -carboxyl-C and α -amino-N of all the amino acids in that chain. The sidechains of the amino acid residues project outside the peptide backbone. The carbonyl-O and the amide-H at the two ends of each peptide bond lie on opposite sides of the peptide backbone. The α -NH₂ group of the terminal amino acid residue at one end of the peptide chain and the α -COOH group of the terminal amino acid at the other end remain free; these two ends of the peptide chain are known respectively as the *N-terminal end* and the *C-terminal end*. Primary structures of many proteins have

been worked out; e.g., human insulin, hemoglobin and adrenocorticotropin, bovine glucagon, ribonuclease and chymotrypsin, chicken lysozyme.

Many amino acids of a protein may be replaced by other specific amino acids without affecting its biological activity. These are called *variable amino acid residues*. In contrast, several *invariant amino acid residues* occupy specific positions in the protein molecule and their replacement so disturbs the primary structure as to affect the biological activity.

II. Higher orders of structure

In an aqueous medium, a peptide chain assumes a specific coiled, folded or zigzag form, orienting and concealing most of its nonpolar amino acid sidechains in the nonaqueous interior of its folds or coils and exposing many of its polar sidechains on the outer surface of the folds or coils. Such a three-dimensional form of the protein constitutes the higher orders of its structure. Evidently, this three-dimensional form depends on the sequence of amino acids in the peptide chain, i.e., on its primary structure. However, amino acid residues, located far apart from each other in the peptide chain, are brought close to each other by such three-dimensional coils or folds of the higher orders of structure. Following types of bonds, mostly noncovalent, are then formed between the amino acid residues, thus closed up. These bonds serve to hold and stabilize the coils and folds of the three-dimensional form of the peptide or protein.

(i) *Hydrogen bonds*: These are weak, low-energy, noncovalent bonds formed by the sharing of a single hydrogen by two covalently bound and strongly electronegative atoms like O and N. The hydrogen bond may be considered as the electrostatic attraction between the hydrogen nucleus of one group and the electronegative atom of another group. Its bond energy is as low as 3-7 kcal mol⁻¹, i.e., about 10% or less of the energy of average

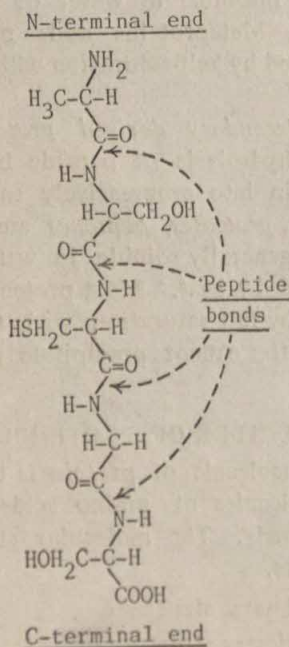


Fig. 3.20. Primary structure of a peptide.

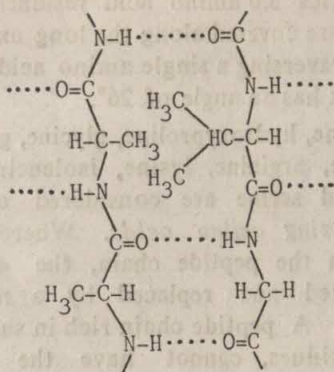


Fig. 3.21. Hydrogen bonds in peptides.

covalent bonds. Hydrogen bonds may be formed in a peptide chain between the α -carbonyl and α -amide groups of different peptide bonds, or between the sidechain carboxyl group of one amino acid residue and the hydroxyl, imidazole or amide group in the sidechain of another amino acid residue (Fig. 3.21). Hydrogen bonds may be destroyed by pH changes, moderate rise in temperature, and concentrated solutions of urea or guanidine hydrochloride. Although individually weak, hydrogen bonds have considerable collective strength, when present in large numbers and at frequent intervals.

(ii) *Ionic or electrostatic bonds*: These are weak noncovalent bonds of energy as low as $3\text{--}7\text{ kcal mol}^{-1}$. Ionic bonds are formed by the electrostatic attraction between the

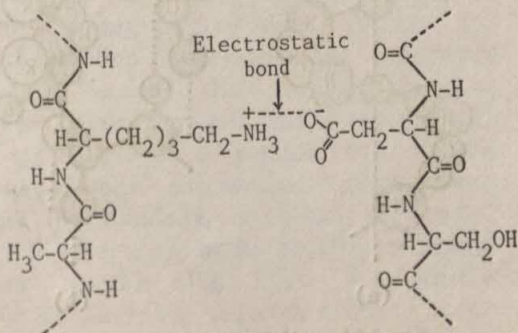


Fig. 3.22. Ionic bond between amino acid sidechains of peptides.

anionic polar sidechain of an amino acid such as glutamate or aspartate, and the cationic polar sidechain of another amino acid such as lysine or arginine (Fig. 3.22). pH changes may alter or destroy the ionic bonds by changing the ionization of the polar sidechains.

(iii) *Hydrophobic bonds or interactions*: These weak forces are caused by interactions between the nonpolar sidechains of amino acid residues like alanine, valine, leucine, isoleucine, methionine, phenylalanine and tryptophan. In an aqueous medium, the water-repellant nonpolar sidechains close up by squeezing out water molecules separating them. Consequently, they occupy the smallest possible volume of the nonaqueous interior of the folds in the peptide chain. The nonpolar sidechains are then held together due to the positive entropy changes from local rearrangements of water molecules, and also the van der Waals forces between the nonpolar groups. The resulting attractive force is called the hydrophobic bond or interaction. Hydrophobic interactions weaken at low temperature.

(iv) *Van der Waals forces*: These are very weak forces of attraction between both polar and nonpolar sidechains of amino acid residues, and possess bond energies like $1\text{--}2\text{ kcal mol}^{-1}$. They result mainly from vibratory and rotatory effects of closely located atoms, and from interactions between oscillating dipoles of adjacent molecules. Van der Waals forces as well as hydrophobic interactions are unaffected by pH changes.

(v) *Covalent disulfide bonds*: An S-S bond may be formed between the sidechain SH groups of cysteine residues in the same or different peptide chains (Fig. 3.23).

The higher orders of protein structure may be formed at three levels, viz., secondary, tertiary and quaternary structures.

1. *Secondary structure*: In an aqueous medium, the peptide chain first assumes a

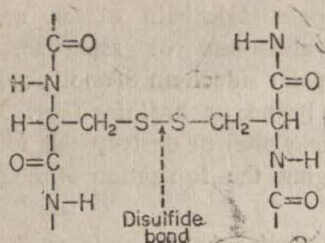


Fig. 3.23. An S-S bond between amino acid sidechains of two peptides.

three-dimensional secondary structure consisting of a helically coiled, zigzag linear or mixed form. The secondary structure results from the steric relationship between amino acids located relatively near each other in the peptide chain. It is held and stabilized by hydrogen bonds between the carbonyl (C=O) and amide (NH) groups of peptide linkages in the peptide chain. *Fibrous proteins* have three main types of secondary structure.

(a) *Alpha helix*: α -Keratins are abundant in hair, nail, wool and the stratum corneum of skin. The molecule is arranged into a regular coil called α -helix. The peptide chain forms regular right-handed helical coils. These coils are stabilized by hydrogen bonds between the carbonyl-O and the amide-N of different peptide bonds, located on the same peptide chain but separated from one another by four amino acid residues (Fig. 3.24). Each peptide bond of the peptide chain is involved in hydrogen bonding. The α -helix gives place to a nonhelical segment wherever regular hydrogen bonding is interrupted. The amide-N, the carbonyl-O and the amide-H, involved in each hydrogen bonding, lie almost along a straight line nearly parallel to the long axis of the helix. The backbone of the coil is identical with that of the primary structure. While the α -C, the α -carboxyl-C and the α -amino-N of each amino acid residue lie on the backbone of the coil, the sidechains or R groups project radially from the backbone. Each turn of the coil has a pitch of 540 pm

and carries 3.6 amino acid residues. About 150 pm are covered along the long axis of the coil in traversing a single amino acid residue. The helix has an angle of 26° .

Proline, hydroxyproline, glycine, glutamate, aspartate, arginine, lysine, isoleucine, threonine, and serine are considered as *helix-destabilizing amino acids*. Wherever they occur in the peptide chain, the α -helix is interrupted and replaced by a nonhelical segment. A peptide chain rich in such amino acid residues, cannot have the α -helical secondary structure at all. The inflexible rings of proline and hydroxyproline, the absence of any α -imino hydrogen in their residues for hydrogen bonding with a carbonyl-O, the large nonpolar sidechain of isoleucine, the electrostatic forces between the charged polar sidechains of glutamate, aspartate, lysine and arginine, and the capacity of β -(OH) of serine and threonine to form hydrogen bonds—all these prevent α -helix formation.

(b) *Beta pleated sheet*: β -Keratins form the fibres of silk, spider's web and reptilian

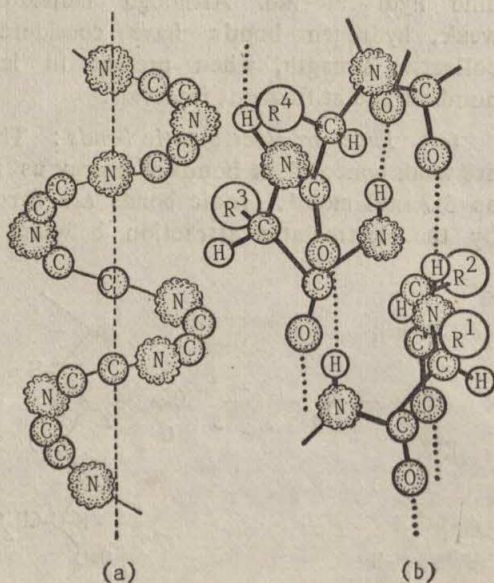


Fig. 3.24. α -Helix. (a) Backbone. (b) Hydrogen bonds.

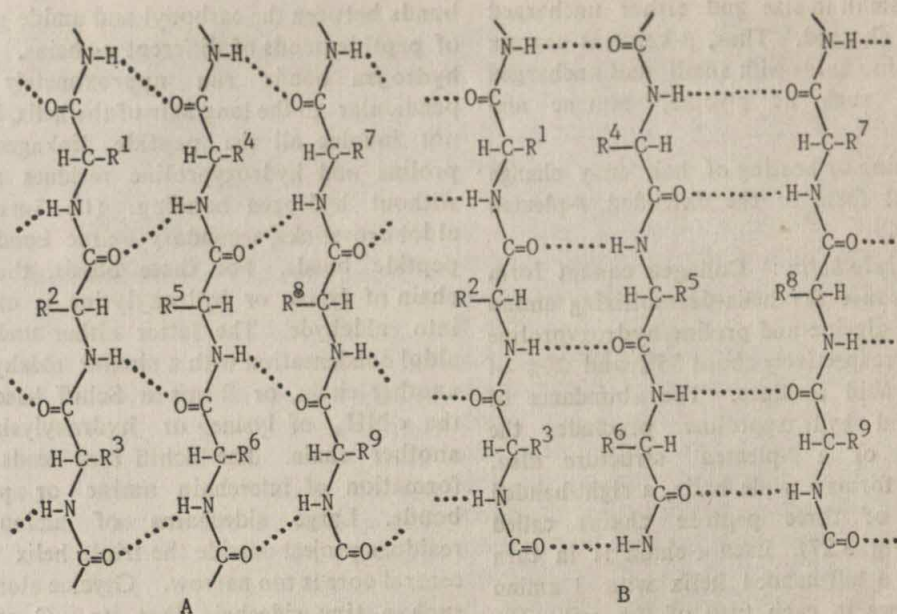


Fig. 3.25. (A) Parallel and (B) antiparallel beta pleated sheets.

claw. The molecule is arranged into non-helical, extended and zigzag linear strands, held closely side by side by hydrogen bonds between the amide-N and the carbonyl-O of peptide linkages of adjacent strands. Unlike the α -helix, the hydrogen bonds of this β -pleated sheet run perpendicular (not parallel) to the long axis of the strands. Similar to the α -helix, all peptide bonds of a chain are involved in hydrogen bonds. The hydrogen bonding of 2.5 such strands of the peptide forms a zigzag pleated sheet. Proline and hydroxyproline interrupt the β -pleated structure because their residues have no α -imino hydrogen left for bonding with the carbonyl-O of a peptide bond. Native β -keratins form *antiparallel* β -pleated sheets where the amide-N, α -C and carbonyl-C occur in reverse orders in two adjacent strands of the peptide (Fig. 3.25). In a *parallel* β -pleated sheet, the sequence of those atoms is identical in interconnected adjacent strands. Many proteins have segments of both parallel and antiparallel β -pleated structures. The

sidechains of amino acid residues project alternately on the two sides of the peptide backbone and consequently lie on the two sides of the zigzag sheet (Fig. 3.26). Several β -pleated sheets are close-packed face to face in a β -keratin fibre. The close cross-linking and packing of the peptide chains in the β -pleated sheet require that the sidechains projecting on the two sides of each sheet

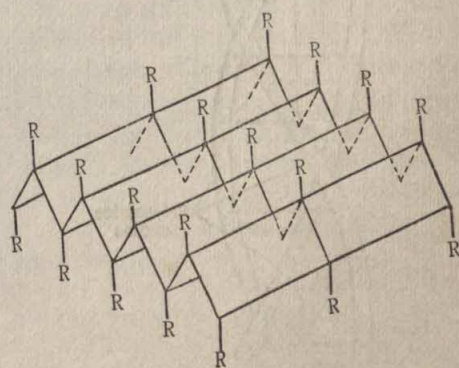


Fig. 3.26. Disposition of R sidechains in beta pleated sheet.

must be small in size and either uncharged or slightly charged. Thus, β -keratins contain mainly amino acids with small and uncharged sidechains such as glycine, alanine and serine.

Stretching or heating of hair may change its α -helical form to the extended β -pleated sheet form.

(c) *Triple helix*: Collagen cannot form α -helix because the helix-destabilizing amino acids, viz., glycine and proline/hydroxyproline constitute respectively about 35% and 20% of its amino acid residues. The abundance of proline and hydroxyproline precludes the possibility of a β -pleated structure also. Instead, it forms a triple helix, a right-handed supercoil of three peptide chains called α -chains (Fig. 3.27). Each α -chain is in turn coiled into a left-handed helix with 3 amino acid residues in each turn of the coil. The triple helix is stabilized by both noncovalent and covalent bonds: (i) Interchain hydrogen

bonds between the carbonyl and amide groups of peptide bonds of different α -chains. These hydrogen bonds run approximately perpendicular to the long axis of the helix, but do not involve all the peptide linkages. So, proline and hydroxyproline residues remain without hydrogen bonding. (ii) Interchain aldol crosslinks, secondary amine bonds and peptide bonds. For these bonds, the side-chain of lysine or hydroxylysine is oxidized into aldehyde. The latter either undergoes aldol condensation with a similar aldehyde of another chain, or forms a Schiff base with the ϵ -NH₂ of lysine or hydroxylysine of another chain. The Schiff base leads to the formation of interchain amine or peptide bonds. Large sidechains of amino acid residues project outside the triple helix whose central core is too narrow. Glycine alone has such a tiny sidechain that its α -C remains buried in the interior of the narrowest part of the triple helix.

Globular proteins like chymotrypsin, myoglobin and ribonuclease have mixed secondary structures instead of uniform structures—different segments of the peptide chain may assume α -helical and/or β -pleated forms with intervening non-helical random coils.

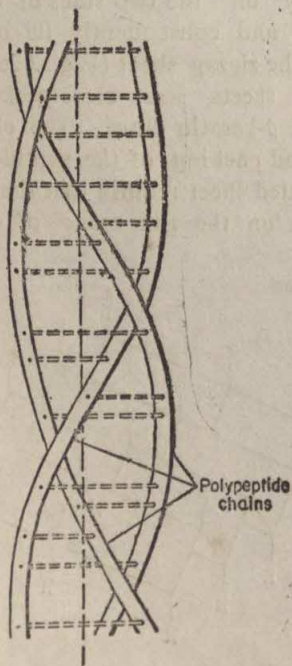


Fig. 3.27. Triple helix.

2. *Tertiary structure*: The peptide chain with its secondary structure may be further folded and twisted about itself, forming many *domains* or structural coils of diverse sizes and shapes, and closing up some distant amino acid residues (Fig. 3.28). A tertiary structure is thereby constituted by the steric relationship between the amino acids, located far apart in the peptide chain but closed up by the folds or coils of the chain. Tertiary structures are held and stabilized in following ways: (i) *Hydrophobic interactions between nonpolar sidechains* of amino acid residues such as alanine, valine, leucine, methionine, isoleucine and phenylalanine. They constitute the major stabilizing forces for tertiary structures. They help to form

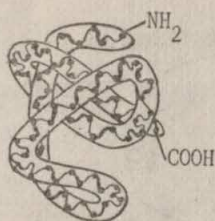


Fig. 3.28. Tertiary structure of a globular protein.

compact three-dimensional folds in the peptide chain by holding nonpolar sidechains close to each other in the interior of the folds. (ii) Hydrogen bonds *between polar sidechains* of different amino acid residues, e.g., between the sidechain COOH of glutamate or aspartate and the imidazole-N of histidine. (iii) Ionic bonds *between oppositely charged polar sidechains* of different amino acid residues. (iv) Van der Waals forces between nonpolar sidechains, or between the sidechains of serine residues or of phenylalanine and tyrosine. (v) Disulfide bonds between the sidechain SH groups of distant cysteine residues, initially brought close to each other. These strong S-S bonds stabilize the tertiary structure, only after it has nearly approached its final form.

3. Quaternary structure: *Monomeric proteins* like ribonuclease and myoglobin bear a single peptide chain and possess only primary, secondary and tertiary structures. *Oligomeric proteins* are formed of two or more interconnected peptide chains. For example, the globin part of hemoglobin is formed of two α and two β chains; aspartate transcarbamoylase is made of six catalytic and six regulatory subunit chains. Oligomeric proteins possess an additional quaternary structure depending on the number, type, spatial relationship and primary structure of its peptide chains. The quaternary structure is stabilized by interchain noncovalent bonds or forces like (i) hydrogen bonds and (ii) ionic bonds, both between polar sidechains on the surfaces of tertiary structural folds of

different peptides, (iii) interchain hydrophobic interactions between nonpolar sidechains left out on the surfaces even after tertiary foldings, and (iv) van der Waals forces.

Role of primary structure in higher orders of protein structure: If the monomeric enzyme ribonuclease is treated with a chaotropic agent like an 8M urea solution or a 6M guanidine hydrochloride solution and with a mild reducing agent like β -mercaptoethanol, these agents respectively disrupt hydrogen bonds and disulfide bonds in the protein, change its tertiary and secondary structures, and consequently inactivate it. But almost complete renaturation and reactivation are effected by removing the denaturing agents and re-oxidizing the SH groups with atmospheric O_2 . Again, the oligomeric protein globin of hemoglobin breaks away from heme, dissociates into its peptide chains and loses its quaternary structure as well as activity, when at an acidic pH in acetone. But its peptide chains spontaneously reunite at neutral pH to re-establish the quaternary structure of globin which again binds to heme to form hemoglobin. These evidences show that the primary structure, left undisturbed during denaturation, can spontaneously re-establish secondary, tertiary and quaternary structures on removal of the denaturing agent. Thus, the primary structure, i.e., the amino acid sequence of the peptide chain, determines the higher orders of protein structure. This is because all higher structures depend on the specific sequence of sidechains of the amino acid residues in the peptide. So, once the primary structure is laid down, the higher orders of structure follow spontaneously in the aqueous biological medium.

3.8 SEPARATION OF PROTEINS

Proteins are extracted from tissues by various solvents like water, dilute acids or alkalies, buffer solutions, dilute salt solutions,

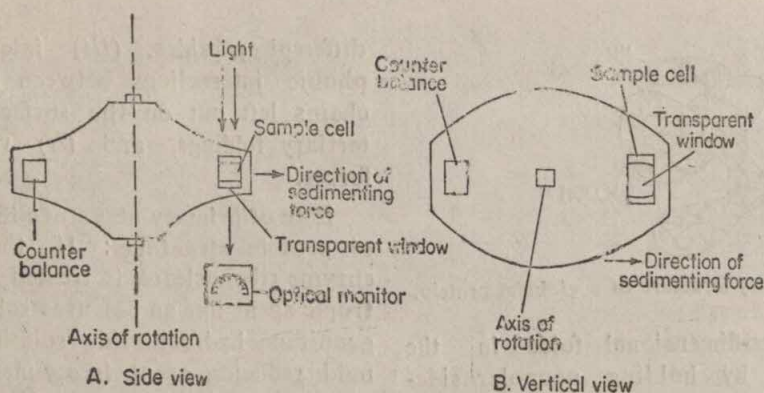


Fig. 3.29. Ultracentrifuge.

grades of alcohol, butanol and acetone. Crude extracts are subjected to methods for the isolation and purification of individual proteins.

1. Ultracentrifugation

On spinning a protein solution in an ultracentrifuge at 40000-70000 rpm, the centrifugal force sediments different proteins at different rates according to the effective mass and diameter of the settling particle, and the difference in density between the particle and the liquid medium.

In ultracentrifugation, the sample is placed in a cylindrical sample cell, with transparent quartz windows, at one end of a rotating arm and is counterbalanced by solvent or water taken in a counterbalance cell at the other end of the arm (Fig. 3.29). Spinning is done at regulated speeds and temperature in a near-vacuum environment inside the ultracentrifuge. The dispersed particles get progressively sedimented during the rotation. Sedimentation velocity (v) is the rate of migration (cm sec^{-1}) of the solute particles in the direction of the centrifugal force.

In *moving boundary ultracentrifugation*, a sharp boundary appears between the solvent and each particle-containing layer, and progressively recedes from the rotational centre. The position of this boundary is determined at intervals for estimating the sedimentation

velocity of the particles of the relevant layer. For this, a beam of light is passed through transparent windows of the sample cell and falls on a photographic film. The blackening of the film is inversely proportional to the concentration of particles in a layer. Alternatively, the transmitted light is received by an optical monitor which determines the change in refractive index at the boundary. A heterogeneous mixture gives as many migrating boundaries as the number of types of homogeneous particles.

The sedimentation velocity v is determined from the outward migration (Δr) of a boundary during an interval Δt . It is expressed in terms of *sedimentation coefficient* (s) which is the sedimentation velocity in cm sec^{-1} per unit field of centrifugal force. Where w is the angular velocity of rotation,

$$s = \frac{\Delta r / \Delta t}{r w^2}; \quad v = \frac{\Delta r}{\Delta t} = s r w^2$$

A sedimentation coefficient of 1×10^{-13} cm sec^{-1} is expressed as one *Svedberg unit* (S). Human IgG and IgM have sedimentation coefficients of 7 S and 19 S respectively. The molecular weight (M) of the sedimenting particles is computed using *Svedberg formula*:

$$M = \frac{sRT}{D(1 - V\rho)}$$

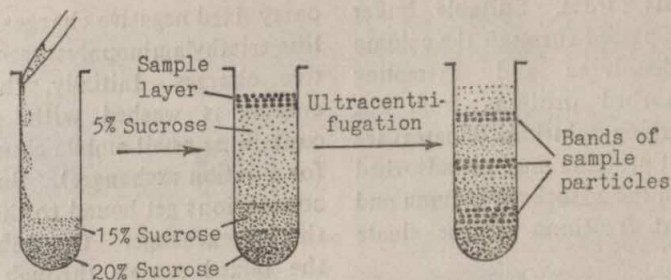


Fig. 3.30. Preformed density-gradient ultracentrifugation.

where R = molar gas constant, T = absolute temperature, D = diffusion coefficient of the solute, V' = specific volume (volume in ml g^{-1}) of the solute, and ρ = density of the liquid.

Preformed density-gradient ultracentrifugation is often used in separating proteins, particularly the protomers obtained by denaturation of an oligomeric protein. Successive volumes of progressively diluter sucrose solutions are poured gently into a plastic centrifuge tube. This establishes a sucrose density gradient rising progressively downwards in the tube. The sample is then gently layered over the upper surface of the column of sucrose solution. On ultracentrifuging the tube, particles of each species of macromolecules descend only upto that layer of the liquid where their density coincides with that of the sucrose solution. Contents of the heterogeneous sample are thus separated into different

layers or bands along the sucrose density gradient (Fig. 3.30). Particles of the highest and lowest densities get concentrated in the lowest and topmost bands respectively. Finally the bottom of the tube is punctured to collect the contents of different bands successively in different tubes.

2. Chromatography

Following chromatographic methods are widely used for separating proteins.

(a) *Adsorption chromatography*: The mixed solution of proteins is passed through a column of solid, fine granules of insoluble adsorbents like silica gel, alumina, charcoal powder, nylon and calcium hydroxyapatite. The solutes are selectively adsorbed on the adsorbent particles. Polar adsorbents adsorb the solutes with the help of ionic or hydrogen bonds. Nonpolar adsorbents adsorb the

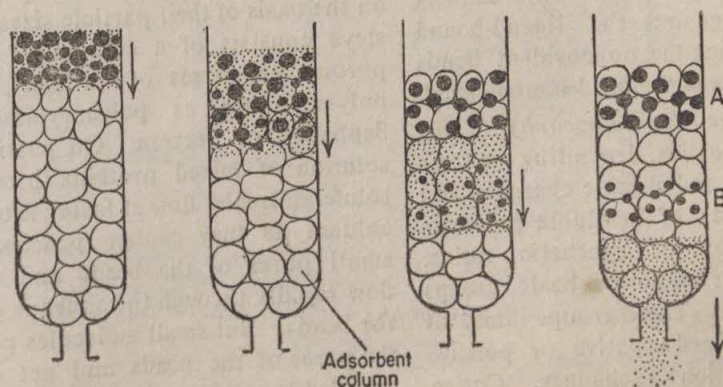


Fig. 3.31. Adsorption chromatography. A,B : separated particles.

solutes by means of hydrophobic interactions or van der Waals forces. Suitable buffer solutions are then passed through the column for selectively dissolving and extracting (elution) the adsorbed proteins from the column. The emerging solutions (eluates) are collected. Different proteins may get adsorbed in different layers of the adsorbent column and emerge in different fractions of the eluate (Fig. 3.31).

(b) *Affinity chromatography*: A biologically active protein is often isolated from a mixture depending on its affinity for binding to some specific molecules (ligands). By suitable chemical methods, the chosen ligand is bound covalently with surface groups of insoluble agarose or dextran beads. For separating an enzyme, the ligand may be its substrate or a substrate analogue, binding specifically with the substrate-binding site of the enzyme. For an antibody, the ligand may be its specific antigen or hapten. When a solution containing the desired protein is passed through a column of the ligand-bound beads, immobilized ligand molecules bind only with the specific protein to keep the latter adsorbed on the beads. But all other proteins pass out through the column. All unbound substances are first washed out from the chromatographic column. Then, a buffer solution of specific pH and ionic strength is percolated through the column. This extracts (eluates) and brings out the ligand-bound protein by dissociating the noncovalent bonds between the protein and the bead-bound ligand.

(c) *Ion exchange chromatography*: This method separates proteins, depending on their acid-base properties and electric charges. Ion exchangers are beads of insoluble polymers like agarose, cellulose or synthetic resins. The beads have either acidic or basic groups fixed on their surface. These groups ionize at suitable pHs to give fixed negative or positive charges on the ion exchanger. *Cation*

exchangers such as sulfonated polystyrene carry fixed negative charges. *Anion exchangers* like triethylaminopolystyrene bear fixed positive charges. Initially, the ion exchanger column is washed with a buffer solution containing small mobile counterions (e.g., Na^+ for a cation exchanger). Some of the mobile counterions get bound to the fixed charges of the ion exchanger. On subsequently passing the mixed sample through the column, the initially bound counterions are exchanged with mobile macromolecular counterions (e.g., protein cations for cation exchangers). The protein ions thus get held electrostatically by the fixed opposite charges of ion exchanger beads. The column is then eluted with buffer solutions of progressively changing pH; this reduces the charges on the retained protein ions and consequently frees them from the fixed charges; the lower the charge of a protein ion, the earlier it is released from the fixed charge. Alternatively, the column is eluted with buffer solutions of rising molarity of small ions; the latter progressively displace and release the protein ions from the exchanger in reverse orders of their charges. In both cases, proteins emerge in different eluate fractions—the higher the charges on a protein, the later is its emergence in the eluate.

(d) *Molecular sieve chromatography or gel filtration*: This method separates proteins on the basis of their particle sizes. Molecular sieve consists of a column of hydrated and porous gel beads of an inert, cross-linked polymer such as polyacrylamide, agarose, Sephadex or dextran. On passing a buffered solution of mixed proteins through it, large solute molecules flow at faster rates down the column as they cannot penetrate into the small pores of the beads and consequently flow rapidly through the aqueous phase outside the beads. But small molecules penetrate into the pores of the beads and get considerably retarded in moving through the column; the

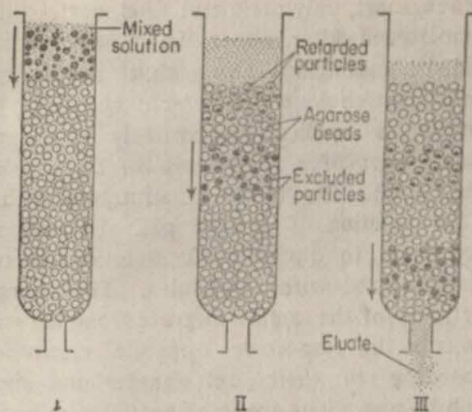


Fig. 3.32. Gel filtration.

degree of retardation is inversely proportional to their particle sizes (Fig. 3.32). Different species of proteins descend as separate bands through the column, in order of their particle sizes. On eluting the column with suitable buffer solutions, larger particles will pass out in earlier eluate fractions while smaller particles will emerge in later fractions.

Polymer beads, having specific pore diameters, may be used also for MW determination of the proteins. Standard proteins of known MWs are first chromatographed through a gel column of specific porosity to

determine their mobilities along the column. The mobility of the unknown protein, when chromatographed through a similar gel column, is compared to those of the standard proteins to estimate the MW of the unknown protein.

3. Electrophoresis

Electrophoresis is the migration of charged solute particles towards the electrode bearing opposite charge when an electric field is created in the solution between two electrodes. The rate of migration of the charged particles depends on (i) the amount of net charge on the solute ion, (ii) the electrical gradient established in the solution, (iii) the size and shape of the solute particles, (iv) the viscosity of the solution and (v) its pH. The pH of the solution may be so adjusted by buffers that different species of proteins bear different amounts and types of net charges. Thus, particles of different proteins migrate at different rates towards the respective opposite electrodes, depending on their charges, sizes and shapes. This separates each homogeneous component from others in a mixture.

(a) *Free-boundary electrophoresis*: Tiselius used this method for fractionating human plasma proteins. The plasma sample is mixed

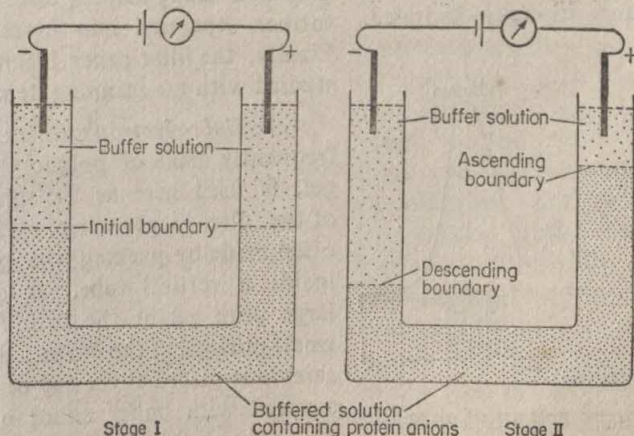


Fig. 3.33. Free-boundary electrophoresis.

with barbiturate buffer (pH 8.6) and the ionic strength is maintained at 0.1. This buffered solution is taken in a flat-faced U-shaped glass cell, immersed in a thermostat bath (Fig. 3.33). Pure buffer solution is layered over the mixed solution in each limb of the cell. An electrical gradient of 6 volts cm^{-1} is established by placing electrodes in the buffer layers of two limbs. As pH 8.6 is more alkaline than the isoelectric pHs of plasma proteins, they exist as anions and migrate towards the anode at different rates. The rate of migration is higher for smaller and more charged protein anions than for larger and less charged ones. Each homogeneous group of protein particles moves in the solution as a separate band forming a moving front or boundary. The refractive index changes sharply at each boundary due to differences in protein concentration across the latter. The positions of the moving boundaries are obtained either by photographic recording of refractive index changes along the limbs of the cell, or from changes in ultraviolet absorbancy. This yields a schlieren or electrophoretic pattern whose peaks indicate the major proteins (Fig. 3.34). The amounts of protein fractions are calculated from the areas under the respective boundaries.

(b) *Paper electrophoresis*: Charged particles may also migrate along a hydrated,

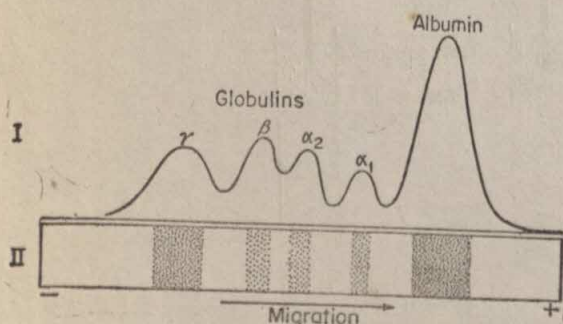


Fig. 3.34. I. Electrophoretic pattern of plasma proteins. II. Stained paper electrophoretic strip.

porous, inert, polymeric and solid matrix like a moistened filter paper when an electrical field is established along that matrix. In *one-dimensional paper electrophoresis*, the sample is applied transversely across the central part of a long strip of filter paper (Fig. 3.35). The latter is saturated with a buffer solution of desired pH. Its ends are then fixed to dip in buffer solutions in two troughs fitted with electrodes. The charged particles of the sample migrate along the strip towards the respective opposite electrodes, according to their net charges and sizes. Each homogeneous group of particles migrates as a separate band whose final location is identified by a suitable staining of the filter paper (Fig. 3.34). The density of staining, measured by a scanning densitometer, is used for estimating the concentration of the component in each band and for converting the bands into corresponding peaks.

For *two-dimensional paper electrophoresis*, the unstained electrophoresed filter paper of the one-dimensional method is sewed into a broader filter paper and electrophoresed again at right angles to the previous direction, using a buffer solution of a new desired pH and a chosen electrical gradient. Particles of each previously separated band may migrate in the direction along the new electrical field and get further separated into more than one zone. Finally, the filter paper is suitably stained and studied with the scanning densitometer.

(c) *Gel electrophoresis*: A gel block, frequently made of polyacrylamide or starch gel, is used here as the solid matrix instead of the filter paper strip. The gel block is often made by packing the gel in a column inside a vertical tube. It often consists of large pore gel at the top of the column and small pore gel at the bottom (Fig. 3.36). The sample is placed at the top of the column and covered with buffer solution. The foot of the column also dips in buffer solution. Electrodes are placed in the top and bottom

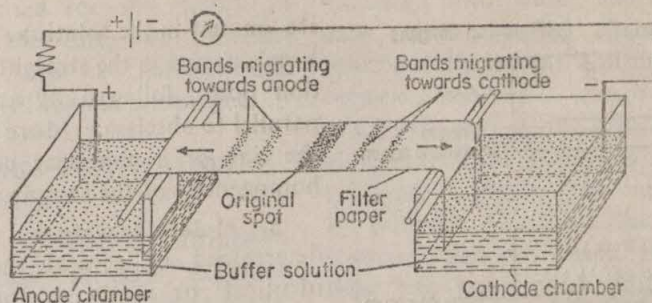


Fig. 3.35. One-dimensional paper electrophoresis.

buffer solutions. Proteins in the sample migrate vertically depending on their charges, sizes and shapes. Thus they get separated in different sections of the gel block. They are then eluted from these sections by buffer solutions. The protein in each collected eluted fraction is assayed.

To determine the molecular weight of a protein, standard proteins of known MWs are first electrophoresed on a gel block of specific porosity. The gel block is then stained with Coomassie blue to determine the location and electrophoretic mobility of the protein bands. The MW of the unknown protein is then estimated by comparing its mobility during electrophoresis on a gel block of the same porosity, with that of the standard proteins.

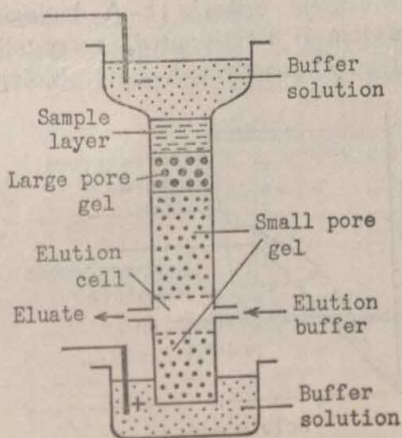


Fig. 3.36. Gel electrophoresis.

(d) *Isoelectric focusing or isoelectrophoresis*: Electrophoresis is done here on a gel block with a stable pH gradient maintained along its length. Each component of the mixed sample migrates in the electric field towards the electrode of opposite charge, but progressively loses the charges and the rate of migration till it reaches the zone of its isoelectric pH. There it forms zwitterions which stop further electrophoretic migration and settle into an immobile band. Thus, all the components are separated as bands at the sites of their respective isoelectric pHs on the gel block. The latter may be stained for studying the bands, or eluted with buffer for analyzing the components of each band. This method simultaneously determines the isoelectric pHs of the separated components.

(e) *Immunoelectrophoresis*: Antigenic proteins are separated by combining their electrophoretic migration with their precipitation by specific antibodies. The sample is placed in the central well of an agar gel block and electrophoresed for $\frac{1}{2}$ -1 hour with an electrical gradient of $3.3 \text{ volts cm}^{-1}$. This causes different antigenic proteins to migrate to different distances along the block (Fig. 3.37). The immune serum (antiserum), containing the antibodies, is then placed in a longitudinal groove of the block, parallel to the line of migration of antigens. The antigens and antibodies are allowed to diffuse through the agar for 18-24 hours. The antibodies consequently react with and

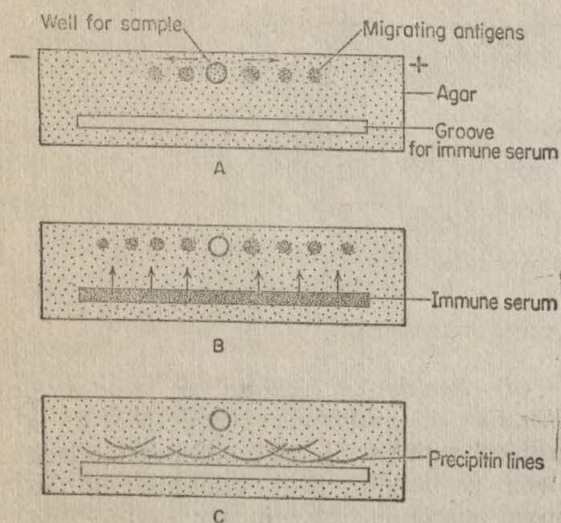


Fig. 3.37. Immunoelectrophoresis.

precipitate the migrating antigens in the form of separate precipitin lines in the gel block. Each homogeneous group of antigen molecules forms a single sharp precipitin line which may be stained.

(f) *Radioimmuno-electrophoresis*: The fluid sample, containing radioactive ^{14}C - or ^3H -labelled antigenic proteins synthesized in tissue cultures, is electrophoresed on an agar block using a specific antiserum till precipitin lines are formed. The block is then overlaid for a period with an X-ray film. The latter is developed after a period of exposure. The film then shows a pictorial reproduction of the precipitin lines, produced on the film by the β particles from the radiolabelled antigens.

3.9 CRITERIA OF PURITY OF PROTEINS

The purity or homogeneity of an isolated protein can be tested as follows.

1. *Solubility curve*: On adding progressively more amounts of a homogeneous solute to the solvent, the amount of solute in solution rises proportionately and produces a sloping straight line in its solubility curve (Fig. 3.38). When the solution gets saturated, further addition of the solute fails to increase

its amount in the solution. So, a single sharp break occurs in the straight line at the saturation point, followed by another straight line parallel to abscissa. More than one break in the line or a nonlinear curve indicates non-homogeneity of the sample.

2. *Molecular weight*: Molecular weights, determined by several methods, should be identical or nearly so for a homogeneous protein.

3. *Ultracentrifugation*: Homogeneity of a protein is indicated in ultracentrifugation by a single sharp moving boundary between the pure solvent and the solute-containing layer, because the homogeneous particles are all driven at the same rate by the centrifugal force. A heterogeneous mixture gives more than one migrating boundary.

4. *Electrophoresis*: In *immunoelectrophoresis* and *radioimmuno-electrophoresis*, each homogeneous protein forms a single sharp precipitin line. In other methods of electrophoresis, each homogeneous protein migrates as a separate band. A heterogeneous mixture forms a number of bands.

5. *Chromatography*: The particles of a homogeneous protein are retained in a single zone of the chromatographic column and emerge in a single eluate fraction.

6. *Biological criteria*: A biologically active protein is fractionated, recrystallized and partially inactivated. Its biological

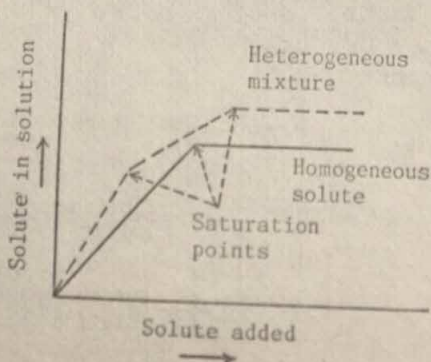


Fig. 3.38. Solubility curves.

effects are then studied for any change in the ratios between the rates of different biological actions. For a homogeneous protein, these ratios will remain unchanged.

3.10 SEPARATION AND PURIFICATION OF AMINO ACIDS

Acid hydrolysis of proteins ultimately produces amino acids through peptides. Proteases like pepsin, trypsin, chymotrypsin, carboxypeptidases, aminopeptidases, and papain may be used to hydrolyze peptide bonds connected to specific amino acids. Bacterial enzymes like Pronase, Nagarse and subtilisin cause more extensive and less specific hydrolysis of proteins.

Amino acids present in a protein hydrolysate may be separated and purified by electrophoresis and chromatography.

1. **Partition chromatography:** Amino acids are separated depending on their distribution ratios or partition coefficients between two immiscible liquids, one forming a *stationary phase* on an inert solid support like filter paper or silica gel, and the other forming a *mobile phase* flowing through that support. This is known as *liquid-liquid chromatography*. For separating amino acids and peptides, following methods are widely applied.

(a) **Paper chromatography:** In *ascending paper chromatography*, a small volume of the test solution is applied near the lower end of a strip of filter paper. This strip remains

saturated with water and is suspended vertically inside a sealed chamber to make its lower end dip into an aqueous solution of an organic solvent in a trough (Fig. 3.39). Due to capillary action, the organic solvent ascends along the filter paper to form the mobile nonaqueous phase; the water held immobile by cellulose fibres of the paper forms the stationary aqueous phase. Partitioned between these two liquid phases, different amino acids move to different distances along the paper according to their partition coefficients between the liquids. After the solvent front reaches near the upper end of the filter paper, the latter is taken out and dried. In *one-dimensional paper chromatography*, the filter paper is then stained with ninhydrin solution. This locates different fractions as separate blue or purple spots. The color densities of the spots are measured for estimating concentrations of different amino acids. In *two-dimensional paper chromatography*, the filter paper is dried after the run of the first organic solvent (A), rotated 90° and chromatographed again using a second organic solvent (B). This further separates heterogeneous fractions, resulting from the first run, along the migration of solvent B (Fig. 3.39)

In *descending paper chromatography*, the filter paper is suspended vertically from the solvent trough. The liquid and the solutes migrate down the paper. One-dimensional or two-dimensional descending chromatography may be used.

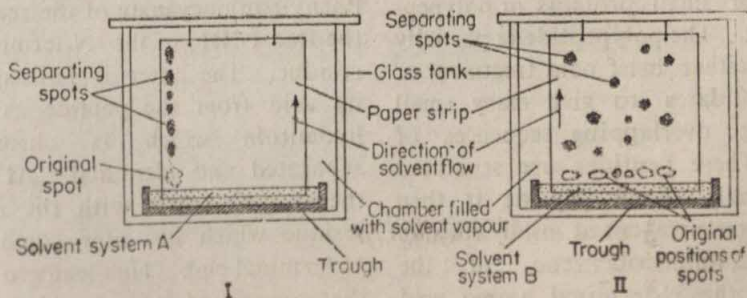


Fig. 3.39. Two-dimensional ascending paper chromatography.

(b) *Thin-layer chromatography*: This method has already been described in connection with the separation of lipids (vide 2.17).

2. *Ion-exchange chromatography*: This method, described for separation of proteins (vide 3.8), may be used in separating amino acids depending on their acid-base properties and charges.

3. *Paper electrophoresis*: As amino acids form cations or anions depending on the pH and their sidechains, they can be separated by paper electrophoresis described for separation of proteins (vide 3.8).

3.11 DETERMINATION OF PRIMARY STRUCTURE OF PROTEINS

Preparatory to the determination of the primary structure, the prosthetic group (if any) is first removed. Hydrogen bonds linking the peptide chains of the oligomeric protein are then cleaved by treatment with denaturing agents like urea or guanidine hydrochloride. Interchain S-S bonds are also broken by oxidation with performic acid or by reduction with β -mercaptoethanol. Thus, the protein is dissociated into its individual linear peptide chains. These are then separated from each other chromatographically or electrophoretically.

The amino acid sequence or primary structure of each peptide chain is next determined by following methods.

1. *Sanger's overlap method*: This is suitable only for small proteins or polypeptides like insulin. The polypeptide is partially hydrolyzed by either brief acid treatment or specific endopeptidases to give many small peptides having overlapping sequences of amino acids. These peptides are separated chromatographically. Each peptide is then treated with Sanger's reagent at mildly alkaline pH. 1-Fluoro-2,4-dinitrobenzene of the reagent changes the N-terminal amino acid residue of each peptide into a deep-yellow

2,4-dinitrophenyl amino acid residue. Each peptide is then totally hydrolyzed into amino acids by prolonged acid treatment and its N-terminal amino acid (released as yellow α -dinitrophenyl amino acid) and other amino acids are separated and identified by chromatography. The N-terminal amino acid and the amino acid composition of all the peptide fragments are then used to find their overlapping sequences. These are used in deducing the amino acid sequence of the original polypeptide.

2. *Edman's automated stepwise method*: This method is suitable for very rapid sequencing of large proteins or polypeptides. A limited number of specific peptide bonds of the protein are cleaved enzymatically or chemically to yield a small number of large peptides bearing 30 or more amino acid residues. Such limited and selective cleavage may be done by using (a) trypsin which specifically hydrolyzes peptide bonds connected to α -COOH of lysine or arginine, (b) cyanogen bromide which specifically cleaves peptide bonds connected with α -COOH of methionine occurring infrequently in the peptide, or (c) *o*-iodosobenzene which specifically cleaves peptide bonds connected to α -COOH of infrequent tryptophan residues. The resulting peptides are separated by chromatography. Each peptide fragment is then sequenced in the spinning cups of an automated apparatus, called *sequenator*. At each step of the process, the peptide is treated with Edman's reagent. Phenylisothiocyanate of the reagent binds with the free α -NH₂ of the N-terminal amino acid residue. The latter is subsequently released by acid from the peptide as a phenylthiohydantoin which is chromatographically separated and identified. At the next step, the reagent reacts with the next amino acid residue which has now come to occupy the N-terminal end. This leads to the release of that amino acid as a new phenylthiohydantoin which is in turn separated and identified. In

this way, all amino acids of the peptide fragment are removed and identified step by step, starting from the N-terminal end. Finally, the primary structure of the protein is worked out by linking the primary structures of its peptide fragments.

3.12 DETERMINATION OF HIGHER ORDERS OF STRUCTURE

1. **X-ray crystallography** : This has been used to determine the secondary and tertiary structures of many proteins like hemoglobin, myoglobin, bovine pancreatic ribonuclease and insulin. On striking a specifically oriented crystal of a protein, X-rays get scattered by the electrons of its atoms. These scattered X-rays produce on a photographic film an X-ray diffraction pattern. The two-dimensional lattice-like form of the latter indicates the distribution of electron densities in the crystal. The diffraction intensifies with the rise in electron density which is higher near the atomic centres than between them. X-ray diffraction patterns are usually taken in several planes using a single crystal of the protein and also an isomorphous crystal of a close derivative differing in respect of a single heavy or

electron-dense metal ion. Electron density maps of the crystal may be drawn by measuring the intensity of the electron-dense spots and their distances from each other in a number of X-ray diffraction photographs. Superimposition of several such maps helps in deducing the three-dimensional molecular conformation of the protein.

2. **Electron microscopy** : Electron micrographs have been used to analyze the quaternary structure of oligomeric proteins. Electron micrographs of the native protein, taken at different planes, are superimposed on each other to construct models of the arrangement of peptide subunits in the protein.

3. **MW determination** : Molecular weight (MW) of the native oligomeric protein is determined during its separation from a heterogeneous sample by ultracentrifugation, gel filtration or gel electrophoresis. Next a mixed sample of all the protomers, obtained by denaturation of the oligomeric protein, is subjected to similar methods for separating the protomers and determining their MWs. These data, coupled with the electron micrographs of the oligomeric protein, are then used for deducing the quaternary structure of the latter.

4. CHEMISTRY OF NUCLEIC ACIDS

Nucleoli, chromatin, ribosomes, chloroplasts and mitochondria are rich in nucleic acids. Transmission of hereditary characters and synthesis of proteins are the primary responsibilities of nucleic acids.

4.1. CONSTITUENTS OF NUCLEIC ACIDS

Nucleic acids are nonprotein nitrogenous substances. Nucleic acids often remain bound to basic proteins like protamines and histones by ionic bonds to form *nucleoproteins*. Nucleic acids are macromolecular polymers of *nucleotides*. A nucleotide is composed of a pentose, a phosphate and a nitrogenous base. The nitrogenous base may be either a purine or a pyrimidine. The pentose is ribose in *ribonucleic acids (RNA)*, and deoxyribose in *deoxyribonucleic acids (DNA)*. Purine bases of nucleic acids consist mainly of adenine (6-aminopurine) and guanine (2-amino-6-oxypurine) and minor amounts of methyladenines, methylguanines, hypoxanthine (6-oxypurine), etc. (Fig. 4.1). Pyrimidine bases of nucleic acids are mainly cytosine (2-oxy-4-aminopyrimidine), uracil (2,4-dioxypyrimidine) and thymine (5-methyluracil) and minor amounts of 5-methylcytosine, 5,6-dihydrouracil, etc. (Fig. 4.2). Symbols for the bases are : A : adenine, G : guanine, T : thymine, C : cytosine, U : uracil, D : dihydrouracil.

Guanine, hypoxanthine, xanthine (2,6-dioxypurine), uric acid (2,6,8-trioxypurine) and all pyrimidines exist in tautomeric enol (lactim) and keto (lactam) forms (Fig. 4.3).

Nucleosides are formed of a nitrogenous base and a pentose. The pentose is β -D-ribofuranose in *ribonucleosides* and β -D-2-deoxyribofuranose in *deoxyribonucleosides*. In *purine nucleosides*, the N⁹ of a purine is linked by the β -N-glycosidic bond with the C¹ of the pentose. In *pyrimidine nucleosides*, the N¹ of a pyrimidine is similarly bound to the C¹ of the pentose (Fig. 4.4).

TABLE 4.1. Some purine and pyrimidine ribonucleosides.

Ribonucleosides	Deoxyribonucleosides
Adenosine (adenine-ribose)	Deoxyadenosine (adenine-deoxyribose)
Guanosine (guanine-ribose)	Deoxyguanosine (guanine-deoxyribose)
Inosine (hypoxanthine-ribose)	
Cytidine (cytosine-ribose)	Deoxycytidine (cytosine-deoxyribose)
Uridine (uracil-ribose)	
Thymidine (thymine-ribose)	Deoxythymidine (thymine-deoxyribose)

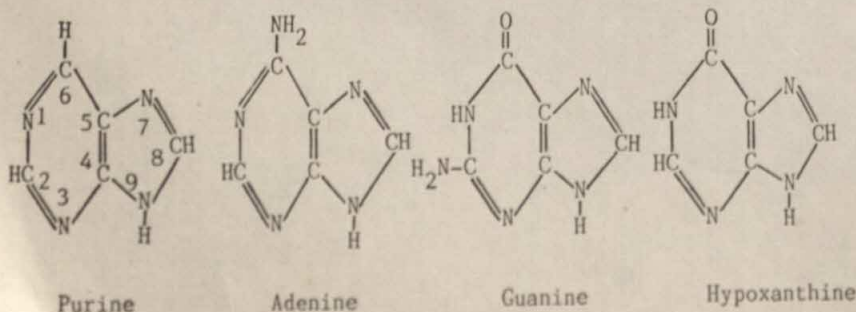


Fig. 4.1. Some purine bases.

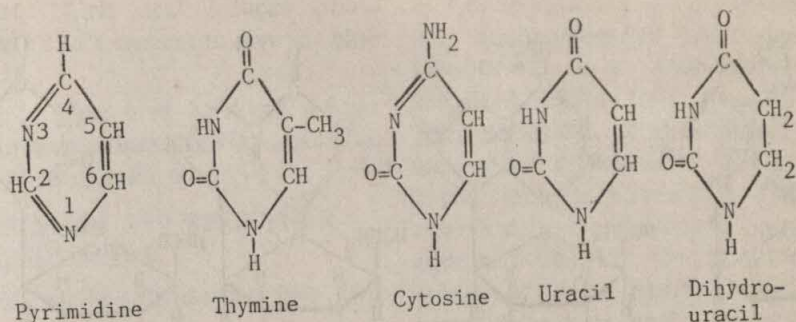


Fig. 4.2. Some pyrimidine bases.

TABLE 4.2. *Some purine and pyrimidine nucleotides.*

<i>Ribonucleotides</i>	<i>Deoxyribonucleotides</i>
Adenylic acid, adenosine monophosphate or AMP (adenine-ribose-P)	Deoxyadenylic acid or dAMP (adenine-deoxyribose-P)
Adenosine diphosphate or ADP (adenine-ribose-P-P)	dADP (adenine-deoxyribose-P-P)
Adenosine triphosphate or ATP (adenine-ribose-P-P-P)	dATP (adenine-deoxyribose-P-P-P)
Guanylic acid or GMP (guanine-ribose-P)	dGMP (guanine-deoxyribose-P)
Guanosine triphosphate or GTP (guanine-ribose-P-P-P)	dGTP (guanine-deoxyribose-P-P-P)
Inosinic acid or IMP (hypoxanthine-ribose-P)	dCMP (cytosine-deoxyribose-P)
Cytidylic acid or CMP (cytosine-ribose-P)	dCTP (cytosine-deoxyribose-P-P-P)
Cytidine triphosphate or CTP (cytosine-ribose-P-P-P)	
Uridylic acid or UMP (uracil-ribose-P)	Deoxythymidylic acid or dTMP (thymine-deoxyribose-P)
Uridine diphosphate or UDP (uracil-ribose-P-P)	
Uridine triphosphate or UTP (uracil-ribose-P-P-P)	Deoxythymidine triphosphate or dTTP (thymine deoxyribose-P-P-P)

Nucleotides are nucleoside phosphates in which one or more OH groups of the pentose residue remain bound to phosphates by ester bonds. According to the presence of ribose or deoxyribose, they may be *ribonucleotides* or *deoxyribonucleotides* (Fig. 4.5).

Nucleotides may be 5'-nucleotides (e.g., adenosine 5'-monophosphate or 5'-adenylic

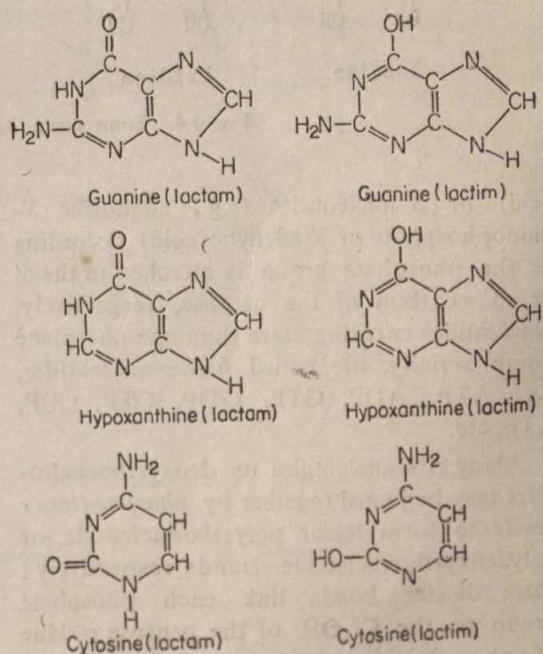


Fig. 4.3. Tautomers of some purines and pyrimidines.

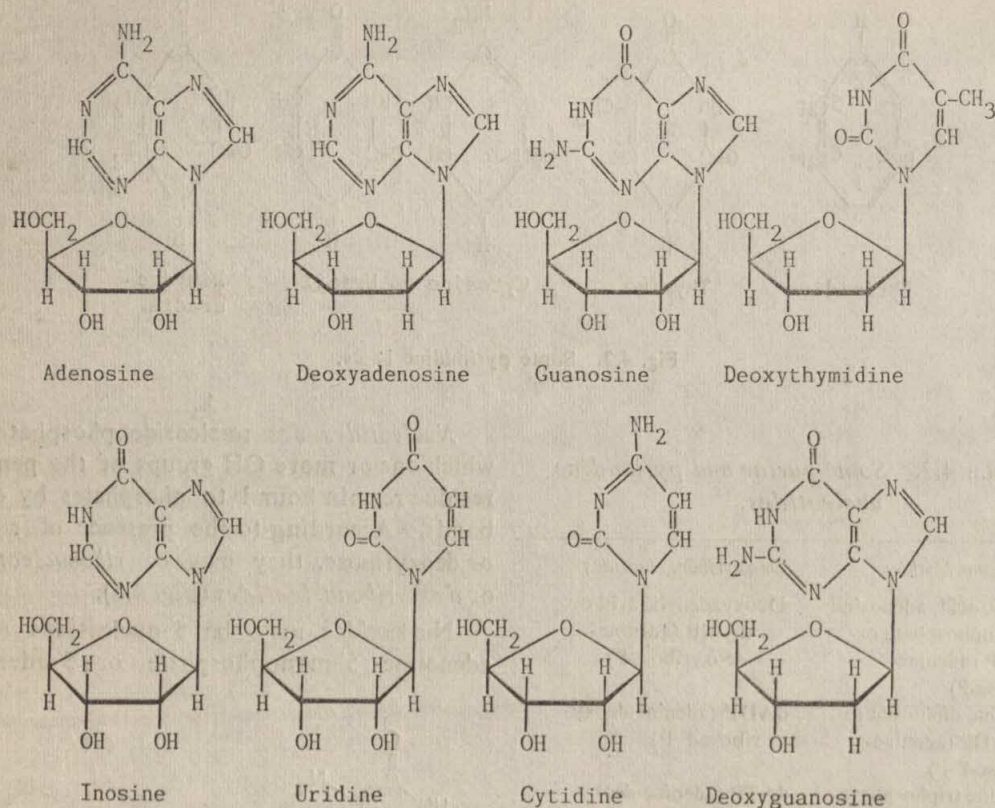


Fig. 4.4. Some purine and pyrimidine nucleosides.

acid) or 3'-nucleotides (e.g., adenosine 3'-monophosphate or 3'-adenylic acid) according as the phosphate group is attached to the 5' or 3' carbon of the pentose, respectively. Nucleotides carrying more than one phosphate group serially, are called *higher nucleotides*, e.g., ATP, ADP, GTP, GDP, CTP, CDP, UTP, etc.

Many ribonucleotides or deoxyribonucleotides may be joined together by *phosphodiester bonds* to form linear polyribonucleotide or polydeoxyribonucleotide strands respectively; these diester bonds link each phosphate group to the C³-OH of the pentose residue of one nucleotide and also to the C⁵-OH of the pentose residue of the next nucleotide (Fig. 4.6).

Properties of uric acid : Uric acid (2,6,8-trioxypurine) is a purine. It occurs in the urine as an endproduct of purine catabolism. It forms white crystals, insoluble in water but soluble in dilute alkalis. It is a weak acid and forms salts like sodium urates. Some tests for uric acid are mentioned below.

Murexide test : On heating uric acid or urates with HNO₃, uric acid is oxidized to urea and alloxan. The latter subsequently changes into alloxantin. On treatment with dilute ammonia, alloxantin changes to a water-soluble purple compound, ammonium purpurate or murexide; addition of dilute NaOH, on the other hand, gives a blue color due to the formation of sodium purpurate.

Folin's test: Uric acid reduces photungstate of Folin's reagent to give a blue color.

Schiff's test: Uric acid blackens a filter paper soaked in ammoniacal AgNO_3 solution, by reducing Ag^+ to metallic silver.

4.2 CLASSIFICATION AND STRUCTURE OF NUCLEIC ACIDS

Nucleic acids are broadly divided into two classes.

1. **DNA or deoxyribonucleic acids:** They are polymers of deoxyribonucleotides and occur in chromosomes, mitochondria and chloroplasts. The nuclear DNA remains bound mainly to basic proteins called histones.

Chromosomal DNA consists of very long double-stranded DNA molecules (MW $1.6 \times 10^6 - 2 \times 10^9$). Each DNA molecule is a polymer of about 10^{10} deoxyribonucleotides. The nucleotides of each strand are bound to each other by covalent 3',5'-phosphodiester bonds—each such bond is formed by the ester linkages of a single phosphate residue with the 3'-OH (i.e., C³-OH group of the sugar residue) of one nucleotide and with the 5'-OH group of the next nucleotide (Fig. 4.6). This forms a linear polydeoxyribonucleotide strand. That end of the strand which bears a free 5'-phosphate group without any phosphodiester linkage to another deoxyribonucleotide, is called the 5' end of the strand; the opposite

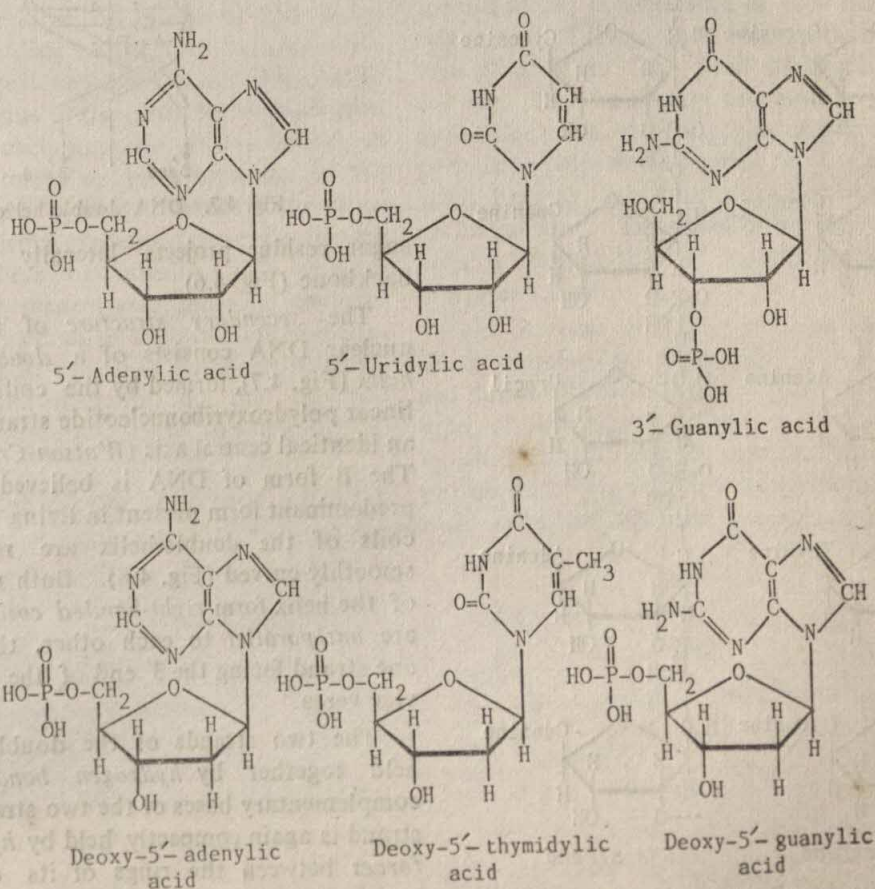


Fig. 4.5. Some purine and pyrimidine nucleotides.

end of the strand bears a free 3'-hydroxyl or 3'-phosphate group and is called the 3' end. The *primary structure* of the DNA molecule is constituted by the number and sequence of different deoxyribonucleotides in its strands. The purine nucleotides in DNA are mainly deoxyadenylic and deoxyguanylic acids while the major pyrimidine nucleotides are deoxycytidylic and deoxythymidylic acids; uracil nucleotides are absent. The backbone of the primary structure is the linear strand of interconnected sugar-phosphate residues while the purine or pyrimidine connected with the

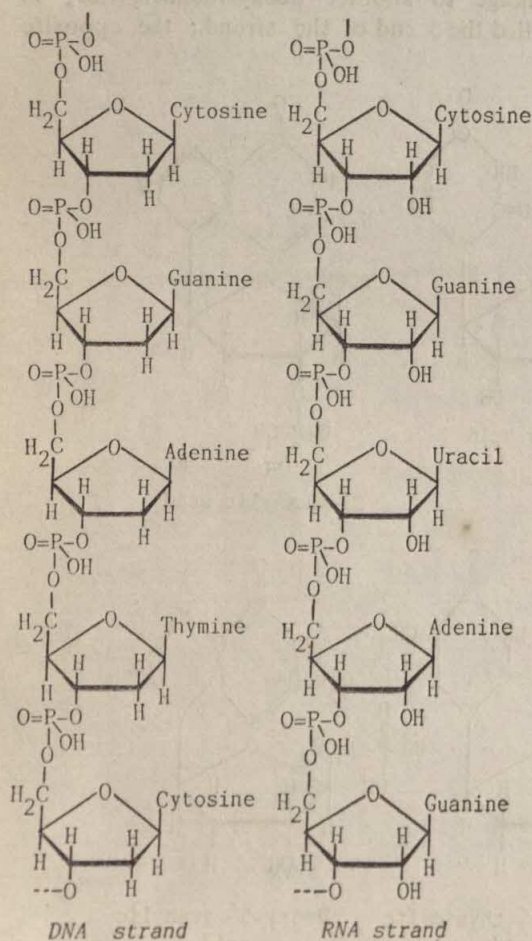


Fig. 4.6. Polynucleotide strands.

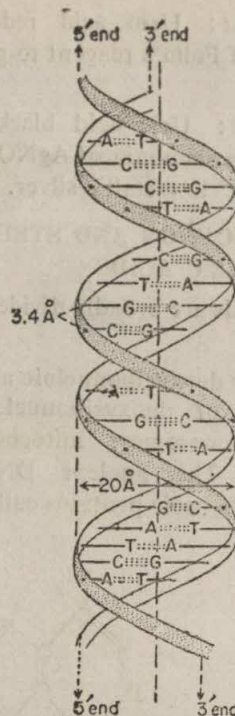


Fig. 4.7. DNA double-helix.

sugar residue projects laterally from that backbone (Fig. 4.6).

The *secondary structure* of B form of nuclear DNA consists of a *double-stranded helix* (Fig. 4.7), formed by the coiling of two linear polydeoxyribonucleotide strands around an identical central axis (*Watson-Crick model*). The B form of DNA is believed to be the predominant form present in living cells. The coils of the double-helix are regular and smoothly curved (Fig. 4.8). Both the strands of the helix form *right-handed coils*, but they are *antiparallel* to each other, the 5' end of one strand facing the 3' end of the other and vice versa.

The two strands of the double-helix are held together by *hydrogen bonds* between complementary bases of the two strands. Each strand is again compactly held by *hydrophobic forces* between the rings of its consecutive bases. The hydrogen bonds bind a purine or

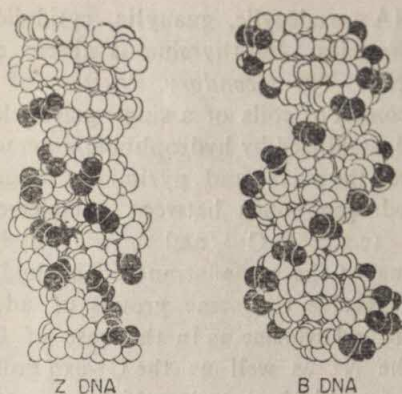


Fig. 4.8. B and Z forms of DNA.

pyrimidine residue of one strand with respectively a pyrimidine or purine of the other strand. According to the *base pairing rule*, each adenine residue of one strand is bound to a thymine residue of the other by two hydrogen bonds while each guanine of one strand is bound to a cytosine or methylcytosine of the other by three hydrogen bonds (Fig. 4.9). Such base pairing explains Chargaff's finding that the numbers of adenine and guanine residues equal respectively those of thymine and cytosine residues in a DNA double-helix. It also follows that purines equal pyrimidines in number in the molecule, and that the two strands are complementary to one another in base sequence.

The phosphodiester backbone of each strand remains towards the outer side of the

coil whose outer surface is formed of the pentose phosphate residues of the nucleotides (Fig. 4.7). Pentose rings lie parallel to the axis of helix. Polar groups of sugar and phosphate residues are mostly exposed to the surrounding aqueous medium. The hydrogen-bonded bases of the two strands lie in the nonaqueous inner core of the double-helix. Two bases of each base-pair face one another edge-on with their planar rings nearly perpendicular to the long axis of the double-helix. The nonpolar rings of purine and pyrimidine bases of each strand tend to be stacked parallel to each other, stabilized by hydrophobic interactions between them. The consecutive hydrogen-bonded base-pairs lie in parallel planes at a distance of 0.34 nm from each other. There are ten base-pairs in each turn of a helix. The pitch of the helix is 3.4 nm. The base-pairs are always purine-pyrimidine pairs. Purine-purine or pyrimidine-pyrimidine pairs do not occur in the double-helix, because their dimensions cannot fit into its central core. The diameter of the double-helix is 1.9 nm; that of its shallow grooves measures 1.2 nm.

The *Z form* of DNA probably occurs in short stretches in drosophila chromosomes and human DNA. It consists of a double-helix of two linear polydeoxyribonucleotide strands. Each strand carries *alternating* purine and pyrimidine deoxyribonucleotide residues such as alternate deoxyguanylate and

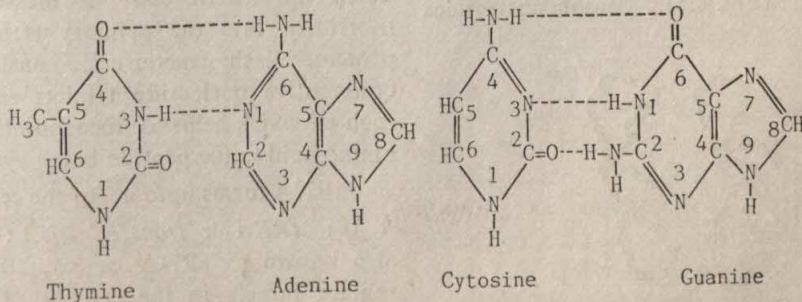


fig. 4.9. Hydrogen-bonding of base pairs.

deoxycytidylate residues, in repeating sequences. Each strand forms a *left-handed coil* around the central axis of the double-helix. Each turn of the helix carries *twelve base-pairs*. The pentose phosphate backbone follows a *zigzag path* along the helix instead of the regular smoothly curved spiral path in B form (Fig. 4.8). The helix has a diameter of 1.8 nm and exists only at high ionic concentrations.

A, C, D and E forms of DNA double-helices may also exist, but carry respectively 11, $9\frac{1}{2}$, 8 and $7\frac{1}{2}$ base-pairs per turn.

Mitochondrial DNA differs from the nuclear DNA in structure. Its individual strands have a primary structure made of only about 10^4 nucleotides. Its secondary structure consists of a double-stranded, endless or closed-loop (circular) molecule which may have a supercoiled tertiary structure (Fig. 4.10).

Prokaryotic bacteria like *E. coli* carry a single chromosome containing a single circular DNA double-helix formed of about 4×10^6 base-pairs.

2. RNA or ribonucleic acids: They are polymers of ribonucleotides and occur in the nucleolus, Nissl granules, ribosomes, mitochondria and cytosol. The *primary structure* of RNA is constituted by the number and sequence of ribonucleotides (i.e., their bases) in the strand. Each linear strand is composed of many ribonucleotides bound to each other by 3',5'-phosphodiester bonds joining the 3'-OH of one nucleotide with the 5'-OH of the next nucleotide (Fig. 4.6). Major nucleotides

in RNA are adenylic, guanylic, cytidylic and uridylic acids. But thymine is absent except in tRNA. The *secondary structure* of RNA may consist of coils of a single polynucleotide strand, stabilized by hydrophobic interactions between the purine and pyrimidine rings, and by hydrogen bonds between complementary bases (e.g., G-C and A-U hydrogen bonding) of the same strand. The hydrogen bonds involve the same groups of adenine, guanine and cytosine as in the case of DNA, and the N³ as well as the C⁴-oxo group of uracil (or dihydrouracil) which pairs with adenine in RNA. Hydrophobic and hydrogen bonds between the bases may also produce a compactly coiled globular *tertiary structure* of RNA.

(a) *mRNA or messenger RNA*: These are relatively large single-stranded fibrous RNA molecules, having MWs of $3 \times 10^4 - 2 \times 10^6$ daltons and carrying $10^3 - 10^4$ ribonucleotides. An mRNA carries adenine, guanine, cytosine and uracil as the major bases along with some minor bases such as methylpurines and methylpyrimidines. The 3' end of most mRNA strands consists of a sequence of about 20-200 adenylate residues (*poly-A tail*). The 5' end of the mRNA strand terminates in a 7-methylguanosine 5'-triphosphate residue (5'-pppG *cap*) bound by a phosphoester bond to the 5'-OH of the 2'-O-methylribose unit of the next ribonucleotide (Fig 4.11).

One or more segments of an mRNA strand carry the *genetic code* or message which is *translated* into the primary structure of a protein. Each genetic code consists of many consecutive nucleotide triplets called *codons*, each of which helps to incorporate a specific amino acid in the peptide being synthesized.

mRNA forms upto 4% of the cellular RNA.

(b) *tRNA or transfer RNA*: These are also known as sRNA or soluble RNA and remain largely in the cytosol. Cellular concentration of tRNA is $\frac{1}{4} - \frac{1}{5}$ th of rRNA. tRNA

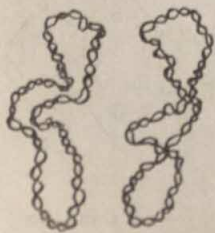


Fig. 4.10. Some forms of mitochondrial DNA.

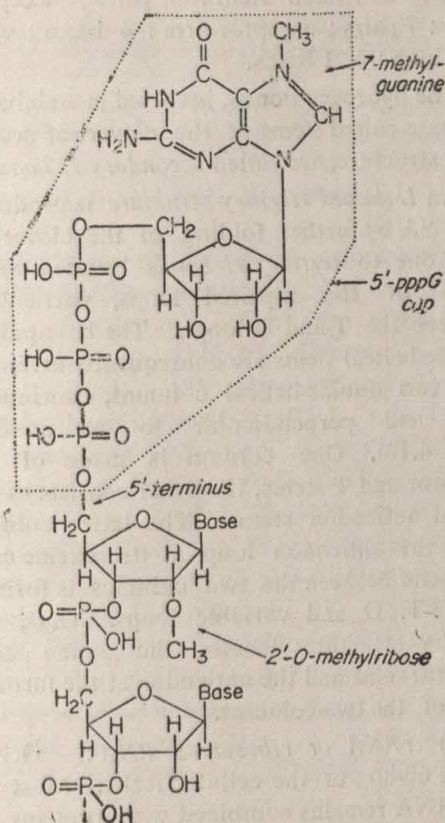


Fig. 4.11. 5'-pppG cap of mRNA.

has relatively small, single-stranded, globular molecules (MW 2.3×10^4). The molecule is a polymer of many ribonucleotides joined by 3',5'-phosphodiester linkages. 20 or more species of tRNA are found in animal cells.

The *primary structure* of each tRNA strand consists of the sequence of ribonucleotides in it. Different tRNAs carry 74 to 95 nucleotides. Their bases include mainly adenine, guanine, cytosine and uracil; pseudouridine (ψ) or uracil 5-ribofuranoside is also present. Thymine occurs in one loop.

Each single-stranded tRNA molecule remains folded to form a *cloverleaf secondary structure* (Fig. 4.12). These folds of the secondary structure are stabilized by hydrogen

bonds between complementary bases in different portions of the same strand; this forms some double-stranded, hydrogen-bonded, [double-helical portions called *stems*. Besides the standard A-U and G-C hydrogen bonding obeying the base pairing rule, hydrogen bonds occur also between slightly mis-matched base-pairs such as guanine and uracil in these stems. Unlike the DNA, planes of the hydrogen-bonded and paired bases are not perpendicular to the long axis of the double-stranded stem and the double-stranded helical portion has a somewhat deformed structure in contrast to the B-DNA double-helix. Some stems bear at one end a *loop* showing no hydrogen-bonding or base-pairing. Some non-bonded single-stranded segments connect the hydrogen-bonded stems with each other.

One of the base-paired stems carries at its farthest end a loop having a sequence of thymine, pseudouridine (ψ) and cytosine. That loop and that stem are called the *T loop* and the *T stem* respectively. An almost diagonally opposite loop carries the dihydrouracil (D) residue; the loop and the stem bearing it are called the *D loop* and the *D stem* respectively.

The 3' end of each tRNA terminates in an unpaired sequence of cytosine-cytosine-adenine. This unpaired arm and its 3' terminal end are called the *acceptor arm* and the *acceptor end* respectively, because the 3'-OH of its terminal adenosine may bind with the α -COOH of a specific amino acid and carry the latter as an amino acyl-tRNA complex for peptide synthesis. The acceptor arm is borne by a base-paired *acceptor stem* whose bases are hydrogen-bonded with the last few bases at the 5' end of the tRNA.

The *anticodon loop* is another unpaired and non-bonded loop carrying a specific sequence of three bases constituting an *anticodon*. The bases of an anticodon may be hydrogen-bonded with three complementary bases of a *codon* (see above) of an mRNA. This helps the tRNA to bring a specific amino acid on a

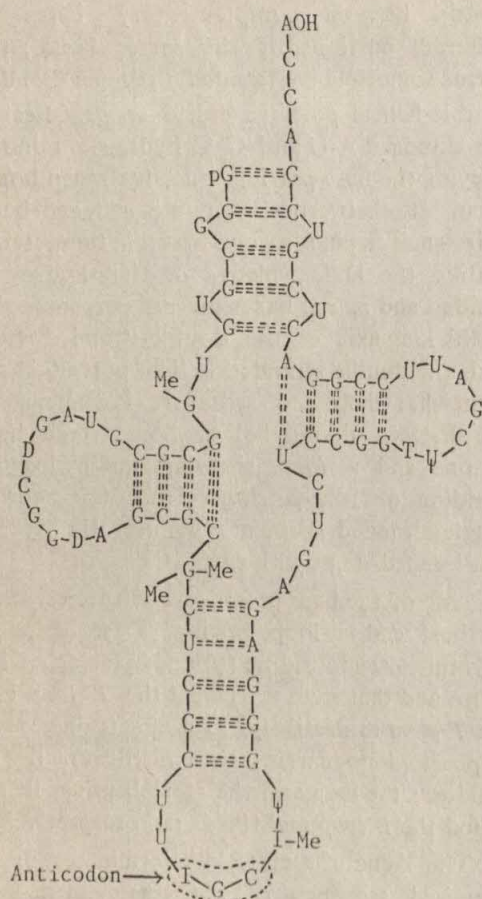


Fig. 4.12. Cloverleaf secondary structure of a yeast tRNA. Me: methyl; I: inosine.

specific codon of mRNA for the incorporation of that amino acid at a particular site of the peptide. The base-paired stem leading to the anticodon loop is called the *anticodon stem*.

A *variable loop* or *extra arm* joins the T stem with the anticodon stem and carries only 4-5 bases in short-chain *class-1 tRNA* (e.g., alanine tRNA), but 13-21 bases in long-chain *class-2 tRNA* (e.g., phenylalanine tRNA).

The number of bases in a loop or stem either remains fixed or varies within a specified range in all tRNAs; e.g., T loop: 7 bases; T stem: 5 base-pairs; D loop: 7-11 bases; D stem: 4 base-pairs; anticodon loop: 7

bases; anticodon stem: 5 pairs; acceptor stem: 7 pairs; acceptor arm: 4 bases; variable loop: 4-21 bases.

The hydrogen bonds, involved in stabilizing the base-paired stems of the cloverleaf secondary structure, are called *secondary H bonds*.

An *L-shaped tertiary structure* is produced in tRNA by further folding of the cloverleaf form due to *tertiary H bonds* between many bases of the unpaired loops, particularly between the T and D loops. The base-paired double-helical stems are consequently arranged into two double-helical columns, continuous with and perpendicular to one another (Fig. 4.13). One column is made of the acceptor and T stems, the other consists of the D and anticodon stems. The latter column holds the anticodon loop at its extreme end. The bend between the two columns is formed of the T, D and variable loops. Thus, the tertiary structure locates the amino acid acceptor end and the anticodon at the furthest ends of the two columns.

(c) *rRNA or ribosomal RNA*: rRNA forms 60-80% of the cellular RNA. Most of the rRNA remains combined with proteins in macromolecular aggregates called *ribosomes*.

Ribosomes, occurring in the cytoplasm and on the cytoplasmic surface of granular

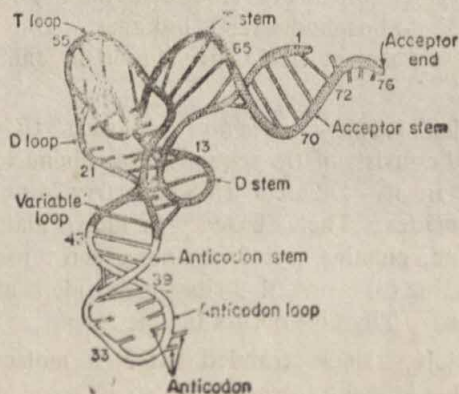


Fig. 4.13. L-shaped tertiary structure of yeast phenylalanine tRNA.

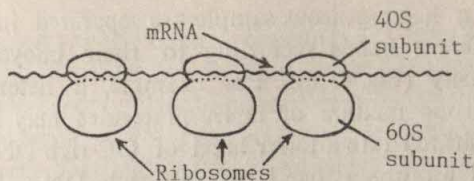


Fig. 4.14. Polyribosome.

endoplasmic reticulum of mammals, possess sedimentation coefficients of 80S and mass of 4.4×10^6 daltons. They are often called *cytoribosomes*. The 80S ribosome is made of a 60S and a 40S ribosomal subunits. The 60S subunit carries 60% rRNA in the form of one each of 28S, 5.8S and 5S rRNA molecules, and 40% protein in the form of about 49 protein molecules. The 40S subunit carries 45% rRNA as an 18S rRNA molecule and 55% protein in the form of about 33 protein molecules. On the granular ER, 5-8 ribosomes are linked by an mRNA strand to form a linear cluster called *polyribosome* or *polysome* (Fig. 4.14). Ribosomes, occurring in mitochondrial matrix, are 55S ribosomes often called *mitoribosomes*. Each of them is made of one 30S subunit containing 12-13S rRNA, and one 40S subunit containing 5S and

16-17S rRNA molecules. Chloroplasts also contain ribosomes smaller than the cytoribosomes. Ribosomes in the cytoplasm of *E. coli* are 70S ribosomes of mass 2.5×10^6 daltons. The *E. coli* 70S ribosome has a 50S and a 30S subunits. The 50S subunit is made of 23S and 5S rRNAs forming 70% of its mass and the remaining 30% consists of proteins. The 30S subunit carries 60% rRNA in the form of a 16S rRNA molecule.

The *primary structure* of rRNA consists of the number and sequence of nucleotides (i.e., their bases) in its molecule. For example, mammalian 5S, 5.8S, 18S and 28S rRNAs are made of about 120, 160, 1900 and 4700 bases respectively. The bases consist mainly of adenine, guanine, cytosine and uracil and a few pseudouridines. Methylated nucleotides occur in low amounts in some mammalian and *E. coli* rRNAs—the methyl group occurs mostly as 2'-O-methyl group of ribose residues and in a few cases, as methyladenine, methylguanine and methylcytosine.

The *secondary structure* of rRNA consists of many short double-helical stems interconnected by single-stranded loops (Fig. 4.15). Each double-helical stem is formed by hydro-

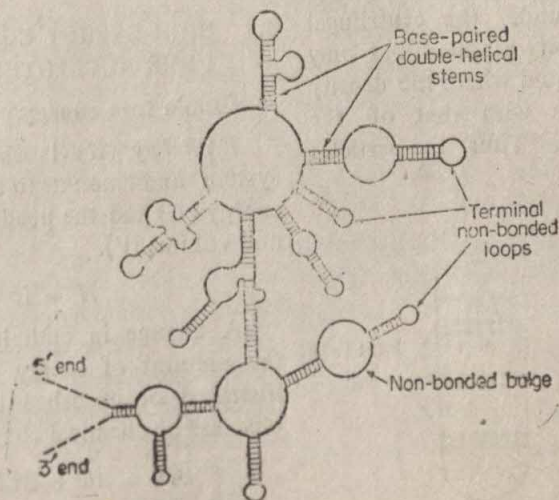


Fig. 4.15. A part of an rRNA molecule. Bars indicate H bonds.

gen bonding between complementary bases in two different stretches of the same rRNA molecule. Each stem has less than 8 base-pairs, bears a non-bonded and unpaired loop at its farthest end, and often carries one or more unpaired bases forming non-bonded bulges (Fig. 4.15).

4.3 SEPARATION OF NUCLEOTIDES AND NUCLEIC ACIDS

Nucleotides may be separated from a mixed sample by thin-layer chromatography and ion-exchange chromatography (vide 2.17 and 3.8).

Nucleic acids may be separated by the following methods.

1. **Density gradient equilibrium sedimentation or isopycnic sedimentation:** The sample is first mixed with a concentrated solution of a salt of a heavy metal such as the chloride or sulfate of cesium. The mixture is then ultracentrifuged for several days. The centrifugal force establishes a downward increasing concentration gradient of heavy metal ions. This results in a continuous liquid density gradient rising progressively in the direction of the centrifugal force. Each homogeneous species of nucleic acid particles descends under the centrifugal force along this density gradient, but only upto that layer of the liquid where the density of the latter coincides with that of the particles of that species. Thus, the particles

of a heterogeneous sample are separated into different bands according to their buoyant density (Fig. 4.16). For example, a heterogeneous mixture of DNA molecules may be separated into a lower band of GC-rich DNA and an upper band of AT-rich DNA by centrifugation at 50000 rpm for 3 days along a CsCl density gradient of $1.65 \rightarrow 1.75 \text{ g ml}^{-1}$. The bottom of the centrifuge tube may then be punctured to collect the materials of separate bands in separate tubes.

2. **Preformed density gradient sedimentation:** In this method of ultracentrifugation (vide 3.8), nucleic acids may be separated along a density-gradient established by layering sucrose solutions of different concentrations successively in the centrifuge tube. A heterogeneous mixture of DNA molecules may be separated into bands of large, medium and small molecules by ultracentrifugation at 50000 rpm for 5 hrs in such a sucrose density gradient.

3. **Gel filtration:** Nucleic acids may also be separated by column chromatography, passing the buffered sample solution through a column of gel beads of specific pore diameters (vide 3.8).

4.4 HIGH-ENERGY COMPOUNDS AND FREE NUCLEOTIDES

Gibb's free energy

Enthalpy (H) is the heat content of a system and amounts to the sum of its internal energy (E) and the product of its pressure (P) and volume (V).

$$H = E + PV$$

A change in enthalpy (ΔH) is a change in the amount of energy in the form of heat liberated or absorbed by the system during physical or chemical changes.

$$\Delta H = \Delta E + \Delta PV = \Delta E + \Delta W$$

where ΔW is the mechanical work done by or on the system.

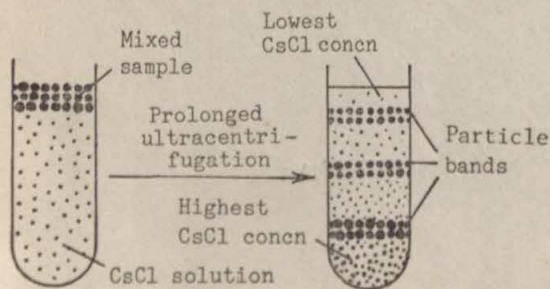


Fig. 4.16, Isopycnic sedimentation

Entropy (S) is the magnitude of randomness or disorder in a system. It is that part of the energy which cannot be transformed into work and is merely spent in creating disorder or randomness in a system. It constantly tends to increase progressively. So, a system left to itself changes spontaneously towards rising entropy until its entropy becomes maximal for the existing state of the system. The latter then attains an equilibrium with maximum randomness and stays as such unless some energy is absorbed by it from its surroundings.

Gibb's free energy (G) is that fraction of the total energy which can perform work or change into an equivalent amount of a new form of usable energy (other than heat) in a system driving towards thermodynamic equilibrium at constant temperature, volume and pressure. The change in free energy (ΔG) of a system is less than the enthalpy change (ΔH) because a part of the latter, amounting to the product of the absolute temperature (T) and the change of entropy (ΔS), is unavailable for work.

$$\Delta G = \Delta H - T\Delta S$$

A negative ΔG , i.e., a fall in the free energy during a process or reaction, involves an increase in entropy. Such a reaction, called an *exergonic reaction*, can take place spontaneously. A positive ΔG indicates that the relevant reaction has to occur in a direction lowering entropy and consequently cannot take place spontaneously. Such a reaction is called an *endergonic reaction*.

Standard free energy change (ΔG°) of a chemical reaction is the change in free energy during the conversion of 1 mole of each reactant to 1 mole of the respective product at 298 K (25°C), 760 mm Hg pressure and 1M concentration of each reactant or product in aqueous solution. ΔG° is the ΔG° at pH 7. Both ΔG° and ΔG° are expressed in J mol⁻¹ or kcal mol⁻¹. ΔG° is given at thermodynamic equilibrium by :

$$\Delta G^\circ = -2.303RT \log \frac{[C] \times [D]}{[A] \times [B]}$$

where R is the molar gas constant, T is the absolute temperature, $[A]$ and $[B]$ are the molar concentrations of reactants, and $[C]$ and $[D]$ are those of products.

An endergonic reaction (positive ΔG°) may be made to occur by coupling it with an exergonic reaction with a larger negative ΔG° , because the two coupled reactions give an over-all negative ΔG° . In such a case, the free energy liberated in the exergonic reaction supplies the energy for driving the coupled endergonic reaction and is largely captured as the bond energy of the products of the latter.

High-energy compounds

Covalent bonds, having a ΔG° of hydrolysis like -7 kcal mol⁻¹ or higher, have been called "high-energy bonds" by Lipmann. Compounds having such bonds are called "high-energy compounds". "High-energy compounds" belong to several classes (Fig. 4.17).

(a) *Phosphoric anhydrides* : They include the higher nucleotides and cyclic nucleotides of purines and pyrimidines ; e.g., ATP, ADP, GTP, CTP, UTP and adenosine 3',5'-cyclic monophosphate (cyclic AMP).

(b) *Thiol esters* like acetyl-coenzyme A ($\Delta G^\circ = -7.7$ kcal mol⁻¹).

(c) *Enol phosphates* like phosphoenolpyruvate ($\Delta G^\circ = -14.8$ kcal mol⁻¹).

(d) *Acyl phosphates* like 1,3-diphosphoglycerate ($\Delta G^\circ = -11.8$ kcal mol⁻¹).

(e) *Phosphoguanidines* like creatine phosphate ($\Delta G^\circ = -10.3$ kcal mol⁻¹).

(f) *Carboxylic-phosphoric anhydrides* like carbamoyl phosphate.

(g) *Sulfuric-phosphoric anhydrides* like adenosine-3'-phosphate-5'-phosphosulfate (active sulfate).

(h) *Sulfonium compounds* like S-adenosylmethionine ($\Delta G^\circ = -10$ kcal mol⁻¹).

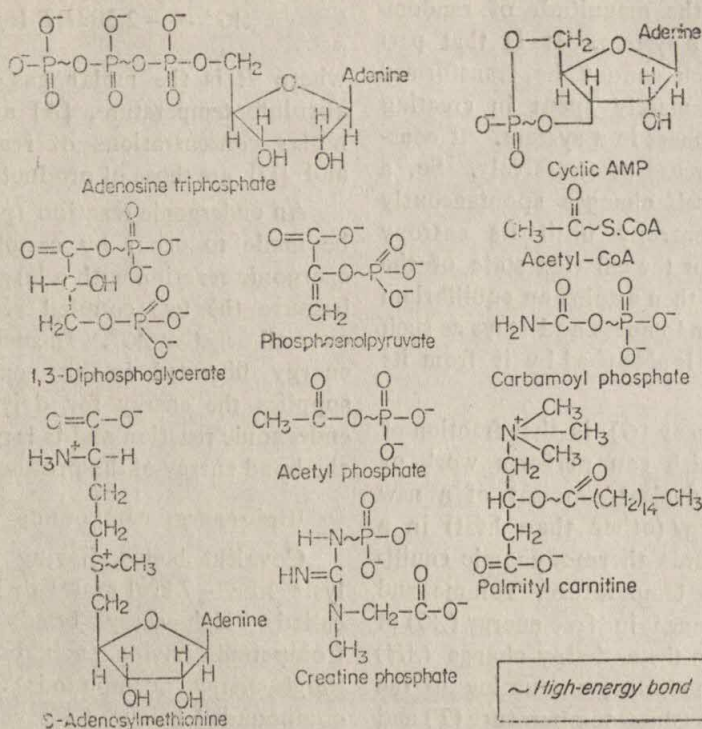


Fig. 4.17. Some high-energy compounds.

The high-energy nature of these compounds arises largely from their (i) *thermodynamic lability* due to the large electrostatic repulsion between anionic groups on two sides of the high-energy bond and a competing resonance resulting from the attraction of electrons from the central group by two groups on two sides of the bond, and (ii) *kinetic stability* due to structural features restricting the exceptionally high resonance.

The energy stored in the high-energy bonds may be utilized in driving many endergonic reactions and processes. Moreover, the group held by a high-energy bond may be transferred to another acceptor molecule along with considerable free energy. Some *high-energy phosphates*, viz., the phosphoric anhydrides such as ATP and GTP, have large negative ΔG° for hydrolysis, lying midway in the order of ΔG° of biomolecules. This

enables them, and ATP in particular, to play key roles in the storage and transfer of energy and in driving endergonic reactions. For example, ΔG° for hydrolysis of ATP to ADP and Pi amounts to about $-8.8 \text{ kcal mol}^{-1}$ and is utilized in transmembrane ion transports against concentration gradients and in many endergonic reactions. The high-energy phosphate of ATP may also be transferred to metabolites such as glycerol and glucose to produce low-energy phosphates like α -glycerophosphate and glucose 6-phosphate with ΔG° of -2.2 and $-3.3 \text{ kcal mol}^{-1}$, respectively. On the contrary, the endergonic synthesis of ATP from ADP and Pi is driven by coupling it with an exergonic reaction of much higher negative ΔG° . Thus, high-energy bonds may be formed in metabolic intermediates like phosphoenolpyruvate and 1,3-diphosphoglycerate which have ΔG° values of -14.8

and $-11.8 \text{ kcal mol}^{-1}$ respectively; these intermediates then transfer their high-energy phosphate to ADP, changing the latter to ATP. ATP or GTP may thus function as *transducers* or intermediate carriers of energy from exergonic to endergonic reactions.

Free nucleotides as high-energy phosphates

ATP or adenosine 5'-triphosphate: It is a high-energy phosphate. Its terminal γ and the next β phosphate bonds are high-energy bonds with ΔG° around $-8.8 \text{ kcal mol}^{-1}$. But the innermost α phosphate bond is a low-energy bond. ATP frequently exists as Mg^{2+} -ATP complex in the tissues and changes into Mg^{2+} -ADP on hydrolysis of its terminal phosphate bond. Energy is mainly held in the body as the phosphate bond energy of ATP. Hydrolysis of ATP into ADP provides the energy for a vast variety of endergonic processes like enzymatic reactions, transmembrane Na^+ transport, muscle contraction and membrane polarization.

ADP or adenosine 5'-diphosphate: Its terminal β phosphate bond is a high-energy bond. But the inner α phosphate bond is a low-energy one. ADP may be hydrolyzed to Pi and adenosine 5'-monophosphate (AMP) which is a low-energy phosphate.

Cyclic AMP or adenosine 3',5'-cyclic monophosphate: It is a high-energy phosphate ($\Delta G^\circ = -11.9 \text{ kcal mol}^{-1}$). It is formed by the cyclization of ATP by *adenylate cyclase*, a membrane enzyme. cAMP has both the 3' and 5' enol groups of its ribose interlinked by phosphodiester bond. cAMP mediates as a *second messenger* in the metabolic actions of many peptide hormones and epinephrine.

Biological roles of other high energy nucleotides such as GTP, GDP, cyclic GMP, CTP, UTP and UDP, are mentioned with relevant reactions in subsequent chapters. Nucleoside triphosphates of purines and pyrimidines serve also as sources for nucleotides incorporated into nucleic acids.

5. ENZYMES

5.1 ENZYMES AS CATALYSTS

Enzymes are colloidal, thermolabile, biological catalysts, protein in nature, and synthesized by living cells. They may catalyze thermodynamically possible reactions either inside or outside the cell. The enzyme may even be extracted from a cell and its activity may remain unimpaired in such a cell-free extract. Substances which may be acted upon by an enzyme are called the *substrates* of that enzyme.

Comparison between enzymes and inorganic catalysts

1. Like inorganic catalysts, enzymes bind with their substrates to form unstable and highly reactive intermediates which subsequently dissociate to give the products.

2. Like inorganic catalysts, enzymes remain unchanged in mass and form, on completion of the reaction. So, very small amounts of an enzyme may be repeatedly used to change large amounts of its substrates.

3. Neither enzymes nor inorganic catalysts can initiate a reaction not occurring spontaneously, at least at a very low rate.

4. Neither inorganic catalysts nor enzymes can alter the equilibrium constant of the reaction; but the equilibrium is attained at a much faster rate in presence of the catalytic agent.

5. Like inorganic catalysts, enzymes act by lowering the *activation energy* required for forming or cleaving covalent bonds.

6. In contrast to small ions or molecules of inorganic catalysts, enzymes are protein macromolecules with specific three-dimensional conformations. Enzyme activity depends on this three-dimensional form of the protein structure.

7. Each enzyme can catalyze a specific reaction of a single or a few specific substrates only. But an inorganic catalyst acts on a large number and a wide variety of substrates and may catalyze diverse types of reactions.

8. Unlike inorganic catalysts, the activity of an enzyme is altered by changes in pH and temperature and on binding with specific substances, because these factors affect its specific three-dimensional form needed for optimum catalysis.

9. In contrast to inorganic catalysts, enzymes are synthesized under the direction of genes and are consequently regulated by factors influencing those genes.

Protein nature

All enzymes are proteins. Some of them are simple proteins while others are conjugated proteins. In the latter, the non-protein part of the molecule is called the *prosthetic group* or *coenzyme*, the protein part is called the *apoenzyme* and the complete molecule, made of the apoenzyme and the prosthetic group, is called the *holoenzyme*. Some hydrolytic enzymes such as ribonuclease of bovine pancreas have a single polypeptide chain in the molecule (*monomeric enzymes*). Many enzymes such as hexokinases, aspartate transcarbamoylase, lactate dehydrogenase and acetyl-CoA carboxylase have more than one peptide chain or subunit in each molecule (*oligomeric enzymes*). Sometimes, several enzyme activities catalyzing different consecutive reactions are located at different sites of the same macromolecule. The latter cannot be fractionated into smaller molecules bearing the individual enzyme activities. Such a macromolecule is considered as a *multi-enzyme system*; e.g., fatty acid synthetase,

pyruvate dehydrogenase, α -ketoglutarate dehydrogenase and glycine synthase. Such a multienzyme system increases the efficiency of the over-all reaction sequence by directly transferring the intermediates from one active site to the next one, thereby avoiding their dilution in the free state. The intermediates remaining bound to the multienzyme system are also protected from diversion to other reactions.

Coenzymes

Some dialyzable and thermostable non-proteins called *prosthetic groups* or *coenzymes* get bound with the enzyme and participate in the enzyme action. Some coenzymes like NADP⁺ bind very loosely and transiently to apoenzymes during catalysis and are released from that combination immediately after the reaction. But other coenzymes such as

FMN, FAD and biotin remain more tightly bound to the respective apoenzymes and do not dissociate from them easily.

The coenzyme often gets changed during the reaction, but is subsequently recovered in the original form. For example, NAD⁺ becomes reduced to NADH while acting as coenzyme for dehydrogenases, but is subsequently reoxidized to NAD⁺. Often, the coenzyme accepts a particular group removed from the substrate or donates a particular group to the substrate. The coenzyme may participate in forming an intermediate enzyme-substrate complex. For example, pyridoxal phosphate, the coenzyme of transaminase, forms a Schiff base intermediate with the substrate amino acid.

Metal ions in enzymes

Many enzymes require metal ions like Ca²⁺, K⁺, Mg²⁺, Fe²⁺, Fe³⁺, Cu²⁺, Zn²⁺, Mn²⁺ and Co²⁺ for their activity. Some of them, called *metal-activated enzymes*, form only loose, easily dissociable complexes with the metal and can easily release the metal without denaturation. *Metalloenzymes* hold the metal tightly in the molecule and do not release it even during extensive purification. Metal ions promote enzyme action by (a) maintaining or producing the active structural conformation of the enzyme (e.g., glutamine synthetase), (b) promoting the formation of the enzyme-substrate complex (e.g., enolase, carboxypeptidase A and pyruvate kinase), (c) causing distortions in the substrate or the enzyme (e.g., phosphotransferases), (d) acting as electron donors or acceptors (e.g., Fe-S proteins and cytochromes), (e) activating nucleophiles or functioning as nucleophiles (e.g., methylmalonyl-CoA mutase and carbonic anhydrase), and (f) forming insoluble complexes with the products to decrease the reverse reaction. Metals (M) may form three types of *ternary complexes* with the enzyme (E) and the substrate (S): *substrate-bridge complexes*

TABLE 5.1. *Examples of coenzymes.*

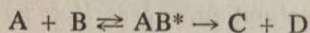
Coenzymes	Some enzymes requiring them
NAD ⁺	α -Ketoglutarate dehydrogenase, pyruvate dehydrogenase
NADP ⁺	Glucose-6-P dehydrogenase, 6-phosphogluconate dehydrogenase
FAD	Succinate dehydrogenase, acyl-CoA dehydrogenase
FMN	NADH dehydrogenase
TPP	Transketolase, pyruvate dehydrogenase, α -ketoglutarate dehydrogenase
Pyridoxal phosphate	Transaminases, AmLev synthase
Tetrahydrofolate	Thymidylate synthase, serine transhydroxymethylase
Coenzyme A	Acyltransferases, thiokinases
Cobamides	Methylmalonyl-CoA mutase, homocysteine methylferase
Biotin	Acetyl-CoA carboxylase, pyruvate carboxylase
Heme	Cytochromes, peroxidases, catalases

(E-S-M), e.g., hexokinases; *metal-bridge complexes* (E-M-S), e.g., enolase; and *enzyme-bridge complexes* (M-E-S), e.g., glutamine synthetase.

Activation energy of enzyme-catalyzed reactions

The *kinetic theory of reaction* proposes that the reactant molecules must collide with or approach each other with a minimum amount of kinetic energy to form bonds. In other words, reactant molecules must collide with sufficient energy to cross the *energy barrier* for the reaction. The minimum energy they must possess to overcome this barrier is called the *activation energy* of the specific reaction.

The *activated complex or transition state theory* considers the activation energy as the difference in energy between the reactants (A, B) and an activated, transient transition intermediate (AB^*). The latter possesses energy above that of the reactants and dissociates into either the products (C, D) or the reactants (Fig. 5.1):



Reactants must gather energy equal to or higher than the activation energy to form the transition intermediate. A higher temperature enhances the reaction rate by increasing the kinetic energy of more molecules above the energy barrier, i.e., by providing the activation energy for forming the transition intermediate.

At the relatively low temperature in living systems, few reactant molecules possess sufficient kinetic energy for effective collisions overcoming the energy barrier. Consequently, the rate of their spontaneous reaction is low. Enzymes bind with their substrates to *lower the energy barrier* by providing alternative reaction paths requiring much less activation energy. Thus, even at a relatively low temperature, the kinetic energy of a larger number

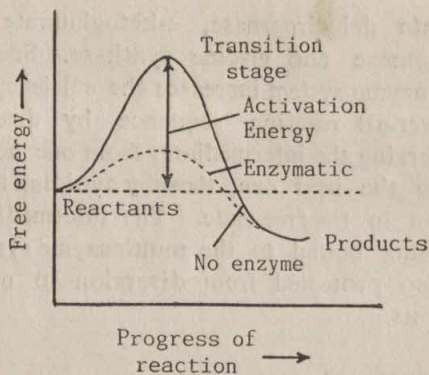


Fig. 5.1. Activation energy of a reaction with and without enzyme.

of reactant molecules suffices for their effective collisions, and the reaction rate is consequently raised.

Reaction equilibria of enzyme-catalyzed reactions

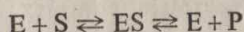
An enzyme *cannot initiate a reaction* which cannot occur spontaneously even at a low rate. Nor does an enzyme alter the *over-all free energy change* of the reaction. It simply enhances the rates of both the forward and reverse reactions by an identical factor. This enables the reaction to attain equilibrium at a much faster rate. But the *equilibrium constant*, i.e., the ratio of rates of the forward and reverse reactions, remains unchanged.

Enzyme-substrate complex

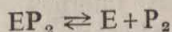
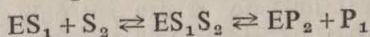
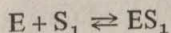
The enzymatic action results from a binding of the reactants with specific sites (*active or catalytic sites*) of the enzyme molecule. This raises local concentration of the reactants far above that of free reactants in the medium, places the relevant bond of the substrate in close and proper alignment with catalytic groups of the enzyme, strains that bond, or provides functional groups of the enzyme for transfer of H^+ and electrons during catalysis.

According to Michaelis and Menten, the enzyme (E) first binds with the substrate

(S) by weak noncovalent bonds to form a transient *enzyme-substrate complex* (ES). The latter soon dissociates into the unchanged enzyme and the product (P). The rate of product formation is proportional to the concentration of the enzyme-substrate complex. Thus, for *single-substrate reactions* such as those catalyzed by isomerases,

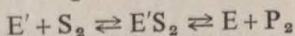
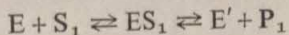


In *single-displacement bisubstrate reactions*, two substrates S_1 and S_2 bind with the enzyme one after another in either a random or an ordered sequence to form a ternary complex (ES_1S_2). The latter eventually dissociates into the enzyme and the products P_1 and P_2 .



Examples : phosphotransferases (e.g., hexokinases), hydrolases (e.g., pepsin), and pyridine-linked dehydrogenases (e.g., lactate dehydrogenase).

In *double-displacement bisubstrate reactions* such as those catalyzed by transaminases, each substrate binds singly with the enzyme in an ordered sequence, forming a binary enzyme-substrate complex. The first enzyme-substrate complex ES_1 dissociates to release the first product P_1 and a modified form E' of the enzyme; the latter then binds with the second substrate S_2 to form $E'S_2$ which subsequently dissociates into P_2 and the original form of the enzyme. No ternary enzyme-substrate complex is formed.



Evidences for ES complex formation: Enzyme-substrate complexes were isolated in some cases (e.g., the ES complex of triose phosphate dehydrogenase). They were detected in some cases by fluorescence spectroscopy (e.g., ES complexes of bacterial tryptophan synthase) or by nuclear or electron magnetic

resonance spectroscopy. ES formation have been optically demonstrated in some cases by either electron microscopy (e.g., nucleic acid polymerases) or X-ray crystallography (e.g., carboxypeptidase A).

Active or catalytic site: The active site of an enzyme is constituted by several amino acid residues, located at some distances from each other in the peptide chain but brought close together by the foldings of secondary and tertiary structures of the enzyme. So, the active site possesses a complex three-dimensional form and provides a predominantly nonpolar cleft or crevice which receives and binds the substrate. Inside this cleft, polar or nonpolar sidechains of some specific amino acid residues bind with specific groups of the substrate while the polar sidechains of some others participate in catalyzing the change in the substrate. A change in the tertiary or quaternary structure may alter the three-dimensional shape of the active site to reduce its substrate-binding and catalytic activities. Some enzymes have been categorized as *serine enzymes*, *lysine enzymes* or *sulphydryl enzymes* according to the essentiality of specific amino acid residues like serine, lysine or cysteine-SH, respectively, at their active sites.

Bonds in ES complexes: ES complexes are formed by mainly noncovalent bonds binding specific groups of the substrate with specific amino acid sidechains at the active site of the enzyme. (a) *Hydrogen bonds* may bind polar groups of a substrate to polar groups of the active site of the enzyme; e.g., hydrogen bonding of a uracil residue of RNA with specific threonine and serine residues of pancreatic ribonuclease. (b) *Electrostatic bonds* may bind ionic groups of a substrate to oppositely charged groups of specific amino acid sidechains of the enzyme; e.g., electrostatic bond between trypsin and its substrate protein. (c) *Van der Waals forces* may bind the enzyme with a substrate possessing steric complementarity in structure

with its active site. (d) *Hydrophobic interactions* between nonpolar groups of the substrate and of the enzyme help to form some ES complexes by providing the binding energy. (e) In contrast, a *covalent bond* binds some enzymes with their substrates; e.g., a Schiff base between the ϵ -NH₂ of a lysine of transaldolase and the ketone group of sedoheptulose 7-phosphate.

Models for enzyme-substrate interaction: According to Fischer's *template or lock-and-key model*, the active site of the enzyme already exists in proper conformation even in absence of the substrate. Thus, by itself the active site provides a rigid pre-shaped template, fitting with the size, form and groups of the substrate. The active site binds with the substrate and catalyzes the reaction without any change in its own three-dimensional structure (Fig. 5.2). This model can explain the specificity of many enzymes for only one of the stereoisomers of the substrate in exclusion of its other stereoisomers (see below). It also fits with the simple substrate saturation kinetics. It can further explain the sequential binding of the coenzyme and substrates with the enzyme. This model proposes that each of those ligands, on binding with the rigid pre-existing template of the active site, provides additional groups for binding the next ligand. But this rigid template model cannot explain the change in enzyme activity in presence of allosteric modulators. *Koshland's induced fit model*, on the contrary, believes in a flexibility of the three-dimensional form of the active site. It proposes

that inspite of the complete primary structure, the active site does not possess the fully active and complementary conformation in absence of the substrate. But either on the approach of the substrate, or during its binding, or following an initial superficial binding, the active site changes its conformation to attain the final catalytic form. This flexibility of the active site may explain complex saturation kinetics, competitive inhibition and allosteric modulation.

Specificity

The enzyme can bind only such substrates as have groups and steric conformations fitting with the specific amino acid sidechains and three-dimensional conformation of the active site. So, it can act only on a limited number of substrates fulfilling such specifications. This is called specificity of enzymes. Many enzymes exhibit *group specificity*. They act only on a specific type of covalent bond associated with specific groups or residues in the substrate. For example, thrombin hydrolyzes only a peptide bond between the α -COOH of arginine and the α -NH₂ of glycine in its substrate fibrinogen.

Many enzymes possess different types of *stereospecificity*. They act only on a specific stereoisomer of the substrate and not on its other stereoisomers. Such stereospecificity results from a *three-point binding* between the substrate and the active site of the enzyme. In other words, three different groups of the substrate bind with three different groups of amino acid residues at the active site of the

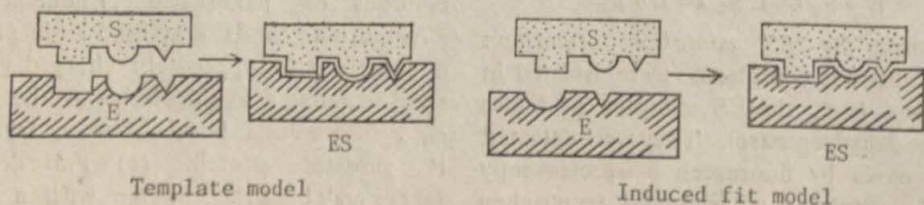


Fig. 5.2. Models for enzyme-substrate interaction.

enzyme. *D-L stereospecificity* is exhibited by D-amino acid oxidase and L-amino acid oxidase which act specifically on the D and L isomers of amino acids, respectively. Many enzymes exhibit *cis-trans specificity* in acting on substrates with double-bonds in the carbon chain. They act specifically on either the *cis* or the *trans* isomer alone of the substrate. For example, fumarase of mitochondria acts only on the *trans* isomer, viz., fumaric acid, and not on its *cis* isomer maleic acid; enoyl-CoA reductase of microsomes acts specifically on Δ^2 -*trans*-enoyl-CoA, and not on Δ^2 -*cis*-enoyl-CoA.

Hormone-induced change in substrate specificity: In nonlactating mammary gland, the enzyme lactose synthetase of Golgi membranes consists of only a catalytic subunit and transfers galactose from UDP-galactose to the oligosaccharide sidechain of a membrane glycoprotein. But prolactin secreted during lactation induces the synthesis of a modifier subunit of the enzyme. Binding of the modifier subunit with the catalytic subunit alters the specificity of the enzyme. It now uses glucose instead of the oligosaccharide as the second substrate and transfers galactose from UDP-galactose to glucose, producing lactose.

Isozymes or isoenzymes

An enzyme may exist in the same organism in several molecular forms. They are distinguished from each other in amino acid composition, electrophoretic property, thermostability, allosteric modulation and immunological property. But all these different proteins catalyze the same reaction with identical substrates. They are called isozymes. Examples: hexokinases I, II, III and IV; lactate dehydrogenases I₁, I₂, I₃, I₄ and I₅. Isozymes of an oligomeric enzyme possess different combinations of its peptide protomers. Different isozymes of an enzyme occur in different tissues or at different locations in

a cell. They differ from each other in kinetic properties such as substrate affinities. Different isozymes may be regulated by allosteric modulators, inducers or repressors in different ways to serve metabolic needs of specific tissues. This differentially regulates metabolic activities in tissues.

5.2 KINETIC PROPERTIES OF ENZYMES

Michaelis-Menten kinetics and substrate concentration

If the substrate concentration is increased progressively from a very low concentration, the enzyme activity at first rises proportionately. This is reflected in an almost linear rise in the initial velocity V . (V is the observed velocity of an enzymatic reaction when very little substrate has reacted). But with further increases in the substrate concentration, the rate of rise in the initial velocity progressively declines. Ultimately at a high substrate concentration, the initial velocity reaches a maximum value (V_{max}) which is not exceeded on further rise in substrate concentration. This is indicated by the rectangular hyperbolic form of the substrate saturation curve obtained by plotting the initial velocity V against the molar concentration $[S]$ of the substrate (Fig. 5.3). Many enzymes obey this *hyperbolic saturation kinetics*. It results from the fact that enzyme is the limiting factor in the enzyme-substrate reaction, because enzyme molecules are far less in number than substrate molecules. So, on gradually increasing the substrate concentration, all enzyme molecules are ultimately saturated with substrates and act at their maximum capacity. A further rise in $[S]$ fails to raise the velocity further due to nonavailability of free enzyme.

Following is the *Michaelis-Menten equation* for a *single-substrate enzymatic reaction* obeying the hyperbolic saturation kinetics:

$$V = \frac{V_{max} [S]}{K_m + [S]}, \text{ or } K_m = [S] \left(\frac{V_{max}}{V} - 1 \right)$$

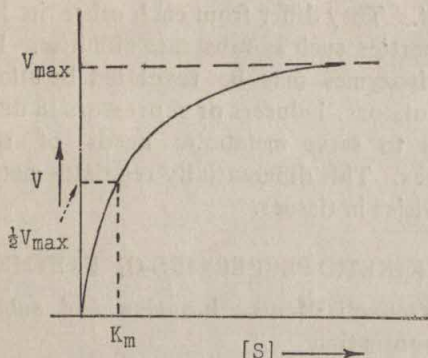


Fig. 5.3. Hyperbolic substrate saturation kinetics.

where K_m is called *Michaelis constant*. When $V = \frac{1}{2}V_{max}$,

$$K_m = [S] \left(\frac{V_{max}}{\frac{1}{2}V_{max}} - 1 \right) = [S]$$

So, K_m is the molar concentration of the substrate at which half the maximal velocity is attained. K_m ranges from about 10^{-6} to about 10^{-1} mole per litre. It indicates the degree of affinity of an enzyme for its substrate. The lower the K_m value, the higher is the substrate affinity of the enzyme. The substrate affinity of an enzyme and consequently its K_m varies from substrate to substrate. For example, pyruvate carboxylase has K_m values of 6×10^{-5} , 4×10^{-4} and 1×10^{-3} M respectively for its three substrates, viz., ATP, pyruvate and HCO_3^- . Isozymes of the same enzyme differ in their K_m values and consequently in their affinities for the same substrate. K_m is not influenced by enzyme concentration or noncompetitive inhibitors, but is changed by competitive and uncompetitive inhibitors, and alterations of pH, temperature and ionic concentration, and in some cases, allosteric modulations.

The initial velocity (V) is directly proportional to the molar concentration $[S]$ of the substrate when the substrate concentration is very low compared to the K_m . In this state, a single-substrate enzymatic reaction becomes

a *first order reaction*, its rate depending on the concentration of the single reactant.

$$[S] \ll K_m, \therefore K_m + [S] \simeq K_m$$

$$\therefore V = \frac{V_{max} [S]}{K_m + [S]} \simeq \frac{V_{max} [S]}{K_m} = K[S]$$

where K is a new constant equaling V_{max}/K_m , because both V_{max} and K_m are constants for a particular enzyme. On the contrary, when $[S]$ far exceeds K_m , the initial velocity attains its maximal value V_{max} and becomes independent of $[S]$. The reaction now turns into a *zero order reaction*, its rate independent of the reactant concentration.

$$[S] \gg K_m, \therefore K_m + [S] \simeq [S]$$

$$\therefore V = \frac{V_{max} [S]}{K_m + [S]} \simeq \frac{V_{max} [S]}{[S]} = V_{max}$$

On inverting the Michaelis-Menten equation,

$$\frac{1}{V} = \frac{K_m + [S]}{V_{max} [S]} = \frac{K_m}{V_{max} [S]} + \frac{[S]}{V_{max} [S]}$$

$$\text{or } \frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

Plotting $1/V$ against $1/[S]$ gives a straight line called *Lineweaver-Burk double-reciprocal plot* (Fig. 5.4). The vertical intercept or y-intercept of the line gives $1/V_{max}$ and its slope equals K_m/V_{max} . The horizontal intercept (x-intercept) of the extrapolated line gives the value of $1/[S]$ corresponding to the zero value of $1/V$ and amounts to $-1/K_m$, because :

$$\text{if } \frac{1}{V} = 0, \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}} = \frac{1}{V} = 0,$$

$$\text{or } \frac{1}{[S]} = -\frac{1}{V_{max}} \times \frac{V_{max}}{K_m} = -\frac{1}{K_m}$$

K_m and V_{max} may be determined graphically from a double-reciprocal plot drawn by plotting the reciprocals of the rates of catalysis, measured at different substrate concentrations, against the reciprocals of the respective substrate concentrations,

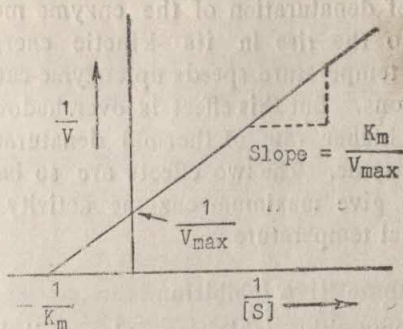


Fig. 5.4. Double-reciprocal plot.

On rearranging the Michaelis-Menten equation,

$$\frac{V}{[S]} = \frac{V_{max}}{K_m + [S]}$$

$$\text{or } \frac{V}{[S]} K_m + V = V_{max}$$

$$\text{or } V = V_{max} - \frac{V}{[S]} K_m$$

Plotting V against $V/[S]$ gives an alternative linear form of the Michaelis-Menten equation, called the *Eadie-Hofstee plot*. The y -intercept of the line gives V_{max} , its slope equals $-K_m$ and its x -intercept is V_{max}/K_m (Fig. 5.5).

The number of substrate molecules, changed into the product every second in a saturating concentration of the substrate, is called the *turnover number* of the enzyme. It equals the rate constant for the reaction breaking ES into E and P , and ranges from less than 1 to $6 \times 10^5 \text{ s}^{-1}$.

For a *double-displacement bisubstrate reaction*,

$$V = \frac{V_{max}}{\frac{K_{m1}}{[S_1]} + \frac{K_{m2}}{[S_2]} + 1}$$

where K_{m1} and K_{m2} are the Michaelis constants for the first substrate S_1 and the second substrate S_2 respectively.

For a *single-displacement bisubstrate reaction*,

$$V = \frac{V_{max}}{\frac{K_{s1} K_{m1}}{[S_1][S_2]} + \frac{K_{m1}}{[S_1]} + \frac{K_{m2}}{[S_2]} + 1}$$

where K_{s1} is the dissociation constant of the ES_1 complex.

Effect of enzyme concentration

At the start of the enzymatic reaction, V is directly proportional to the enzyme concentration. With saturating substrate concentrations, V_{max} is a function of the enzyme concentration. A straight line is obtained by plotting V against the enzyme concentration if the substrate concentration exceeds that for V_{max} , because enzyme is the limiting factor in the enzyme-substrate reaction and providing more enzyme molecules enables the conversion of progressively larger numbers of substrate molecules. Enzyme concentration, however, does not influence the equilibrium constant and K_m .

Effect of product concentration

Reaction products may occupy the active site of the enzyme and make it nonavailable for substrates. Products may also promote the reverse reaction to form the substrate again. So, a rise in product concentration reduces the rate of enzymatic reaction.

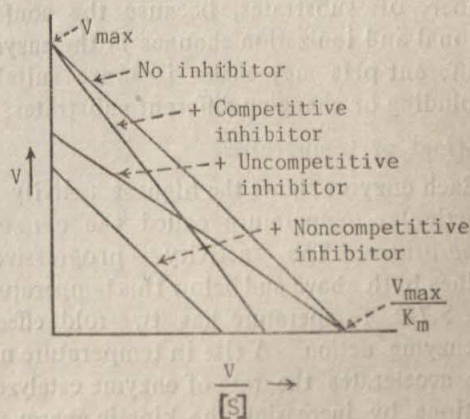


Fig. 5.5. Eadie-Hofstee plots.

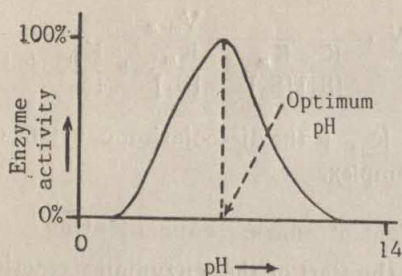


Fig. 5.6. Effect of pH on enzyme activity.

Effect of pH

Each enzyme acts best at a particular pH called the *optimum pH*—the activity declines both above and below that pH (Fig. 5.6). Change in pH (i) may alter the ionization of both the substrate and the active site of the enzyme, (ii) may dissociate the apoenzyme from the prosthetic group, and (iii) may even inactivate the enzyme by disturbing the ionic and hydrogen bonds stabilizing its three-dimensional structure. All these affect the activities of the enzyme. Trypsin, for example, is active in the pH range of 2.5–11, because a functional COO^- group at its active site changes into a nonfunctional COOH group below pH 2.5 while the $\epsilon\text{-NH}_3^+$ group of lysine at the binding site of the substrate changes to a nonbinding NH_2 group above pH 11. The optimum pH may vary from substrate to substrate for enzymes acting on a number of substrates, because the conformational and ionization changes in the enzyme at different pHs may make it more suitable for binding or changing different substrates.

Effect of temperature

Each enzyme shows the highest activity at a particular temperature called the *optimum temperature*. The activity progressively declines both above and below this temperature (Fig. 5.7). Temperature has two-fold effects on enzyme action. A rise in temperature not only accelerates the rate of enzyme-catalyzed reactions by increasing the kinetic energy of the reactant molecules, but also enhances the

rate of denaturation of the enzyme molecule due to the rise in its kinetic energy. A high temperature speeds up enzyme-catalyzed reactions. But this effect is overshadowed by a far higher rate of thermal denaturation of the enzyme. The two effects are so balanced as to give maximum enzyme activity at the optimal temperature.

Competitive inhibition

Competitive inhibition, also called *substrate-analogue inhibition*, is a type of reversible inhibition of the enzyme. The inhibitor binds reversibly with the enzyme to depress or nullify the catalytic action of the enzyme. In competitive inhibition, the inhibitor bears a close structural similarity with the substrate (Fig. 5.8) and competes with the latter for the binding groups of the enzyme. The inhibitor (I) binds with the binding group of the enzyme to form an enzyme-inhibitor complex (EI). So, the substrate affinity of the enzyme is lowered progressively with the rise in the inhibitor concentration. This causes a progressive decline in the velocity of the enzyme-catalyzed reaction. The lowering of the substrate affinity by the competitive inhibitor is indicated by a rise in K_m to $K_m(1 + [I]/K_i)$, where $[I]$ is the molar concentration of the inhibitor and K_i is the dissociation constant of EI. Thus, the

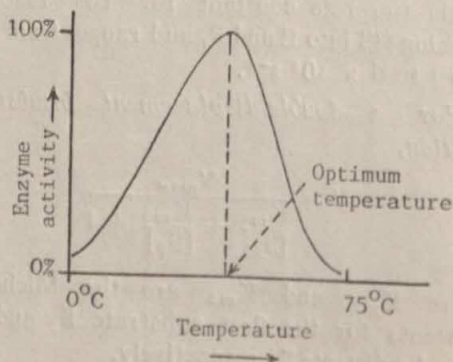


Fig. 5.7. Effect of temperature on enzyme activity.

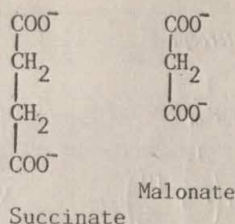


Fig. 5.8. Substrate (succinate) and competitive inhibitor (malonate) of succinate dehydrogenase.

higher the inhibitor concentration, the higher is the apparent K_m of the inhibited enzyme. But a high concentration of the substrate may overcome competitive inhibition by forcing the inhibitor out from its combination with the enzyme. So, a high substrate concentration produces the same maximum velocity V_{max} as in the absence of the inhibitor. The higher the inhibitor concentration, the higher is the substrate concentration necessary for attaining the maximum velocity. The degree of inhibition depends, therefore, on the relative concentrations of the substrate and the inhibitor as also on the relative affinities of the enzyme for the two.

TABLE 5.2. Examples of competitive inhibition.

Enzyme	Substrate	Competitive inhibitor
Succinate dehydrogenase	Succinate	Malonate
Aconitase	cis-Aconitate	trans-Aconitate
Dihydrofolate reductase	7,8-Dihydro-folate	Amethopterin
Diphosphoglycerate mutase	1,3-Diphosphoglycerate	2,3-Diphosphoglycerate
HMG-CoA reductase	HMG-CoA	HMG
Lactate dehydrogenase	Lactate	Oxamate
PP-ribose-P glutamyl amido-transferase	PP-ribose-P	AMP

The Michaelis-Menten equation is modified in presence of a competitive inhibitor :

$$V = \frac{V_{max} [S]}{K_m \left(1 + \frac{[I]}{K_i}\right) + [S]}$$

The equation for the double-reciprocal plot changes accordingly :

$$\frac{1}{V} = \frac{K_m}{V_{max}} \left(1 + \frac{[I]}{K_i}\right) \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

Thus, the double-reciprocal plot of the competitively inhibited reaction has the same y-intercept ($1/V_{max}$) as that of the uninhibited reaction (Fig. 5.9). This indicates that the maximum velocity remains unchanged. But the plot of the inhibited reaction has a steeper slope and an x-intercept of smaller magnitude (Table 5.3). The slope becomes progressively steeper and the x-intercept progressively smaller with the rise in inhibitor concentration.

The Eadie-Hofstee plot of the competitively inhibited reaction shows no change in the y-intercept (V_{max}), but possesses a steeper slope and a smaller x-intercept (Table 5.4 and Fig. 5.5).

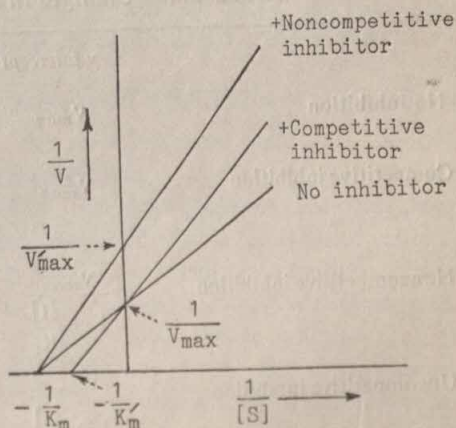


Fig. 5.9. Double-reciprocal plots of competitive and noncompetitive inhibitions.

TABLE 5.3. *Changes in double-reciprocal plot on inhibition.*

	<i>y</i> -intercept	<i>x</i> -intercept	slope
No inhibition	$\frac{1}{V_{max}}$	$-\frac{1}{K_m}$	$\frac{K_m}{V_{max}}$
Competitive inhibition	$\frac{1}{V_{max}}$	$-\frac{1}{K_m \left(1 + \frac{[I]}{K_i}\right)}$	$\frac{K_m}{V_{max}} \left(1 + \frac{[I]}{K_i}\right)$
Noncompetitive inhibition	$\frac{1}{V_{max}} \left(1 + \frac{[I]}{K_i}\right)$	$-\frac{1}{K_m}$	$\frac{K_m}{V_{max}} \left(1 + \frac{[I]}{K_i}\right)$
Uncompetitive inhibition	$\frac{1}{V_{max}} \left(1 + \frac{[I]}{K_i}\right)$	$-\frac{1 + \frac{[I]}{K_i}}{K_m}$	$\frac{K_m}{V_{max}}$

Noncompetitive inhibition

This is another type of reversible inhibition. But here the inhibitor bears no structural similarity with the substrate and binds reversibly with a site of the enzyme other than its substrate-binding site. So, the inhibitor may combine with both the free enzyme and the ES complex. This probably brings about changes in the three-dimensional conformation of the enzyme to produce a failure of catalysis or of the release of the product. In this way, the noncompetitive inhibitor lowers the velocity *V* of the enzyme-catalyzed reaction and the

turnover number of the enzyme. It also reduces V_{max} of the reaction. But the noncompetitive inhibitor does not change the substrate affinity or K_m of the enzyme as it does not occupy the substrate-binding site of the latter. Moreover, because the substrate and the inhibitor bind with different sites of the enzyme, the inhibition cannot be reversed and the original V_{max} cannot be regained by raising the substrate concentration. As the V_{max} is reduced by a factor $(1 + [I]/K_i)$, the Michaelis-Menten equation is changed as follows:

TABLE 5.4. *Changes in Eadie-Hofstee plot on inhibition.*

	<i>y</i> -intercept	<i>x</i> -intercept	slope
No inhibition	V_{max}	$\frac{V_{max}}{K_m}$	$-K_m$
Competitive inhibition	V_{max}	$\frac{V_{max}}{K_m \left(1 + \frac{[I]}{K_i}\right)}$	$-K_m \left(1 + \frac{[I]}{K_i}\right)$
Noncompetitive inhibition	$\frac{V_{max}}{1 + \frac{[I]}{K_i}}$	$\frac{V_{max}}{K_m \left(1 + \frac{[I]}{K_i}\right)}$	$-K_m$
Uncompetitive inhibition	$\frac{V_{max}}{1 + \frac{[I]}{K_i}}$	$\frac{V_{max}}{K_m}$	$-\frac{K_m}{1 + \frac{[I]}{K_i}}$

$$V = \frac{V_{max} [S]}{\left(1 + \frac{[I]}{K_i}\right) (K_m + [S])}$$

The equation for the *double-reciprocal plot* also changes accordingly :

$$\frac{1}{V} = \frac{K_m}{V_{max}} \left(1 + \frac{[I]}{K_i}\right) \times \frac{1}{[S]} + \frac{1}{V_{max}} \left(1 + \frac{[I]}{K_i}\right)$$

Thus, both the slope and the *y*-intercept of the double-reciprocal plot are increased by a factor $(1 + [I]/K_i)$ in noncompetitive inhibition, but the *x*-intercept $(-1/K_m)$ remains unchanged (Fig. 5.9). So, the plot now has a steeper slope and a higher *y*-intercept, both rising progressively with the rise in $[I]$. (Vide Table 5.3).

The *Eadie-Hofstee plot* shows no change in the slope $(-K_m)$ in noncompetitive inhibition (Fig. 5.5), but the *y*-intercept is lowered and the *x*-intercept also declines in value (Table 5.4).

Uncompetitive inhibition

This type of reversible inhibition occurs in many bisubstrate and multisubstrate reactions, but rarely in single-substrate reactions. Here, the inhibitor neither binds with the free enzyme nor inhibits the formation of ES complex. On the contrary, the inhibitor binds with only the ES complex and thereby enhances the substrate affinity of the enzyme to lower its K_m . This inhibits the release of the product from the enzyme-substrate-inhibitor complex and consequently lowers the turnover number, V and V_{max} . The latter cannot be regained by increasing $[S]$. Both V_{max} and K_m are reduced by a factor $(1 + [I]/K_i)$ to change the Michaelis-Menten equation as follows :

$$V = \frac{V_{max} [S]}{K_m + [S] \left(1 + \frac{[I]}{K_i}\right)}$$

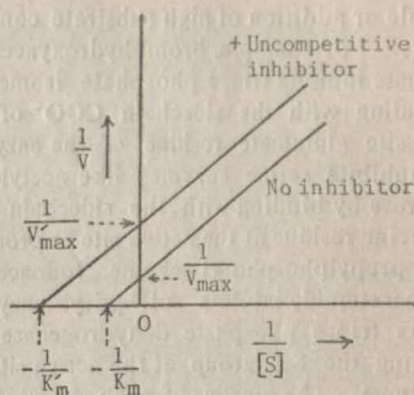


Fig. 5.10. Double-reciprocal plot of uncompetitive inhibition.

The equation for the *double-reciprocal plot* changes accordingly :

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}} \left(1 + \frac{[I]}{K_i}\right)$$

Thus, both the *y*- and *x*-intercepts rise in magnitude by a factor $(1 + [I]/K_i)$, but the slope remains unchanged at K_m/V_{max} (Table 5.3). This makes the plots of uninhibited and inhibited reactions parallel to each other (Fig. 5.10).

The *Eadie-Hofstee plot* of uncompetitive inhibition shows no change in the *x*-intercept (V_{max}/K_m) , but the slope and the *y*-intercept are lowered (Table 5.4 and Fig. 5.5).

Irreversible inhibition

Some inhibitors such as iodoacetate, iodoacetamide, di-isopropylfluorophosphate (DFP) and bromohydroxyacetone phosphate bind covalently with the active site of the enzyme. The resulting complex dissociates into the free enzyme and the inhibitor at only a very slow rate. These inhibitors are structurally different from the substrate. They reduce the enzyme activity *irreversibly*, because they continue to remain bound to the enzyme even after prolonged dialysis of the enzyme-inhibitor mixture, cleavage of the enzyme

molecule or addition of high substrate concentrations. For example, bromohydroxyacetone phosphate inhibits triose phosphate isomerase by binding with the sidechain COO^- of the active site glutamate residue of the enzyme. DFP inhibits serine enzymes like acetylcholinesterase by binding with the sidechain OH of a serine residue at the active site to produce a di-isopropylphosphoryl enzyme. Iodoacetate or iodoacetamide inhibits sulfhydryl enzymes such as triose phosphate dehydrogenase by alkylating the SH group at the active site of the enzyme. The kinetics of such irreversible noncompetitive inhibition resembles that of reversible noncompetitive inhibitions.

5.3 REGULATORY PROPERTIES OF ENZYMES

Irreversible covalent activation

Some strong proteases are secreted as inactive *proenzymes* or *zymogens*. On arrival at the site of activity, one or more specific peptide bonds are hydrolyzed in the pro-enzyme either enzymatically or by pH changes to yield the active enzyme and some inactive peptides called masking substances (Table 5.5). For example, inactive trypsinogen of the pancreatic juice is hydrolyzed to active trypsin in the intestinal lumen by enterokinase of intestinal juice when the intestinal contents have a pH around 5.5. But with the subsequent rise of intestinal pH to about 7.9, already activated trypsin hydrolyzes more

trypsinogen molecules to trypsin. Such activation by the cleavage of some covalent bond is irreversible. The enzyme cannot be reconverted to the original proenzyme.

Reversible covalent modification

Some enzymes are reversibly activated and inactivated by adding or removing phosphate or adenylate groups. For example, a protein kinase of muscle uses ATP to phosphorylate phosphorylase kinase and glycogen synthase, respectively activating and inhibiting them; phosphatases remove hydrolytically the phosphate groups from those enzymes, respectively inactivating and activating them. An adenylyl transferase inactivates glutamine synthase (*E. coli*) by transferring adenylate groups to it from ATP; a deadenylylase activates glutamine synthase by removing the adenylate groups from it through phosphorolysis.

Allosteric modulation and sigmoid kinetics

Activity of some enzymes is enhanced or inhibited by the binding of specific low-MW regulatory metabolites with a specific site of the enzyme, called the *allosteric site*. Such regulatory substances are called *allosteric effectors*; they may be allosteric activators or inhibitors according as they enhance or reduce the enzyme activity by binding with the allosteric site. Following evidences show that the allosteric site is different from the

TABLE 5.5. Activation of proenzymes.

Proenzyme	Activator	Products	
		Enzyme	Masking substance
Pepsinogen	HCl/pepsin	Pepsin	Peptides
Trypsinogen	Enterokinase/trypsin	Trypsin	Hexapeptide
Pro-rennin	HCl	Rennin	Peptide
Prothrombin	Prothrombinase	Thrombin	Polypeptide
Plasminogen	Fibrinolysokinase	Plasmin	Peptide
Procarboxypeptidase A	Trypsin	Carboxypeptidase A	Peptides

substrate-binding site of the allosteric enzyme:

(i) Allosteric effectors can sometimes protect the active site of the enzyme from denaturation even though the substrates are ineffective in preventing the denaturation. (ii) Mutations, radiations, mercurials, proteases, and changes in pH or temperature may frequently change the allosteric property without altering the catalytic activity of the enzyme. In oligomeric enzymes, the allosteric site and the active (substrate-binding) site are located on different protomer chains. The allosteric site possesses specificity for binding only specific allosteric effectors. Separate allosteric sites may exist in an enzyme for allosteric activators and inhibitors. Changes in the enzyme-substrate interaction due to the allosteric effects of regulatory molecules other than the substrate, are called *heterotropic allosteric modulations*. Some examples of such allosteric modulations are cited in Table 5.6. Allosteric activators and inhibitors exhibit respectively positive and negative cooperativities with the substrates.

TABLE 5.6. Some examples of allosteric modulation.

Enzymes	Allosteric activators	Allosteric inhibitors
Acetyl-CoA carboxylase *	Citrate	
Aspartate transcarbamoylase *		CTP
Carbamoyl phosphate synthase *	PP-ribose-P	UTP
Fructose diphosphatase *		Fructose di-P, AMP
Glutamate dehydrogenase †	ADP	ATP, NADH
Hexokinase *		Glucose 6-P
Isocitrate dehydrogenase †	ADP	ATP
Phosphofructokinase *	Fructose 6-P, AMP, FDP	Citrate, ATP
PP-ribose-P synthase *		AMP, ADP, GMP, GDP
Protein kinases *	Cyclic AMP	
Pyruvate carboxylase †	Acetyl-CoA	ADP

* Cytoplasmic enzymes. † Mitochondrial enzymes.

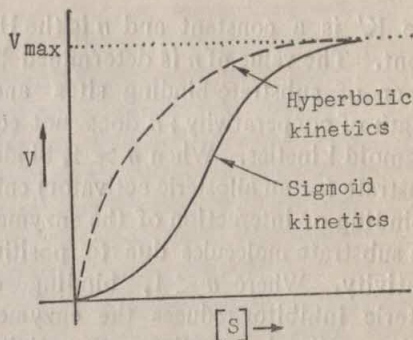


Fig. 5.11. Sigmoid saturation curve of allosteric enzyme compared with hyperbolic saturation curve.

An allosteric enzyme carries more than one substrate-binding site on its different protomer chains. Binding of a substrate molecule with one active (substrate-binding) site produces conformational changes in the enzyme to promote the binding of the next substrate molecule to another active site of the same enzyme molecule. There is thus a positive cooperativity between the substrate molecules of an allosteric enzyme. When the binding of a substrate molecule enhances the interaction between the allosteric enzyme and more molecules of the same substrate, it is called a *homotropic allosteric effect*.

Allosteric enzymes show the *sigmoid saturation kinetics* instead of the Michaelis-Menten kinetics. In case of such enzymes, the plotting of the initial velocity V of the reaction against the molar concentration $[S]$ of the substrate gives a sigmoid curve (Fig. 5.11). This shows that V rises at a low rate with the rise in $[S]$ when $[S]$ is at low levels; but V rises much more steeply at higher levels of $[S]$ producing a steeper slope of the curve above its inflection. This results from the *cooperative binding* of the substrate with multiple sites of the enzyme.

The *Hill equation* for the sigmoid kinetics is as follows :

$$V = \frac{V_{max} [S]^n}{K' + [S]^n}$$

where K' is a constant and n is the Hill coefficient. The value of n is determined by the number of substrate-binding sites and the strength of cooperativity; n does not equal 1 in sigmoid kinetics. When $n > 1$, binding of a substrate (or an allosteric activator) enhances the binding or interaction of the enzyme with more substrate molecules due to positive cooperativity. Where $n < 1$, binding of an allosteric inhibitor reduces the enzyme-substrate interaction by negative cooperativity.

On inverting the Hill equation,

$$\frac{1}{V} = \frac{K' + [S]^n}{V_{max} [S]^n}, \quad \text{or} \quad \frac{V}{V_{max} - V} = \frac{[S]^n}{K'}$$

$$\text{or} \quad \log \frac{V}{V_{max} - V} = n \log [S] - \log K'$$

Thus, plotting $\log [V/(V_{max} - V)]$ against $\log [S]$ gives a straight line of slope n (Fig. 5.12). When $V = \frac{1}{2}V_{max}$, $\log [V/(V_{max} - V)] = \log 1 = 0$. So, the substrate concentration for $\frac{1}{2}V_{max}$ corresponds to that against 0 value of $\log [V/(V_{max} - V)]$ in sigmoid kinetics.

Allosteric inhibitors shift the substrate saturation curve to the right; the curve is shifted to the left in presence of allosteric activators. Allosteric enzymes are divided into K and M series according to their kinetics. In *K* enzymes (e.g., aspartate transcarbamoylase and phosphofructokinase)

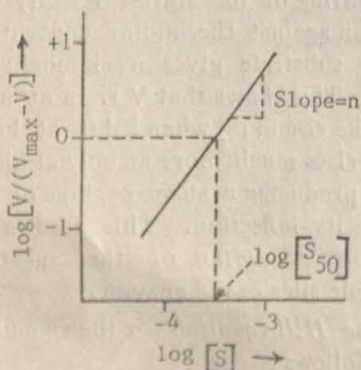


Fig. 5.12. Logarithmic plot of Hill equation.
 $[S_{50}]$ = substrate concentration for $\frac{1}{2}V_{max}$.

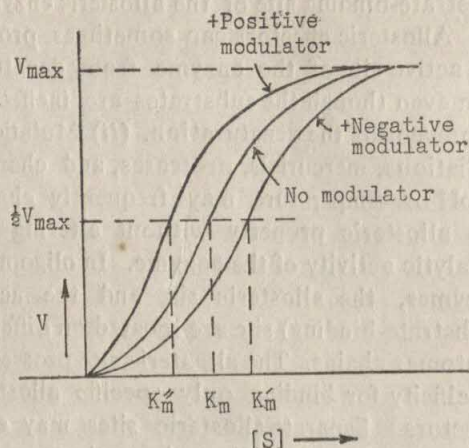


Fig. 5.13. Substrate saturation curves of K series of allosteric enzymes. Positive modulator=activator; negative modulator=inhibitor.

the allosteric inhibitor lowers the substrate affinity to raise the K_m of the enzyme. But the maximum velocity V_{max} remains unaltered (Fig. 5.13). In *M* enzymes (e.g., acetyl-CoA carboxylase), the allosteric inhibitor reduces the maximum velocity V_{max} , but does not change the K_m or substrate affinity (Fig. 5.14). Allosteric activators produce a fall in K_m in *K* enzymes and a rise in V_{max} in *M* enzymes.

Often the final product of a metabolic pathway allosterically inhibits an enzyme for an earlier step of that pathway (*feedback allosteric inhibition*); e.g., the pyrimidine nucleotide CTP allosterically inhibits aspartate transcarbamoylase, an enzyme for pyrimidine synthesis. A metabolite may also cause *feed-forward allosteric activation* of an enzyme for a subsequent step of its metabolism; e.g., fructose 1,6-diphosphate allosterically activates pyruvate kinase catalyzing a subsequent step of its metabolism in glycolytic pathway.

Cyclic AMP acts as an allosteric activator of protein kinases in muscle. Binding with allosteric subunits of the protein kinase, cAMP causes the dissociation of the enzyme into a cAMP-allosteric subunit complex and free active catalytic subunits.

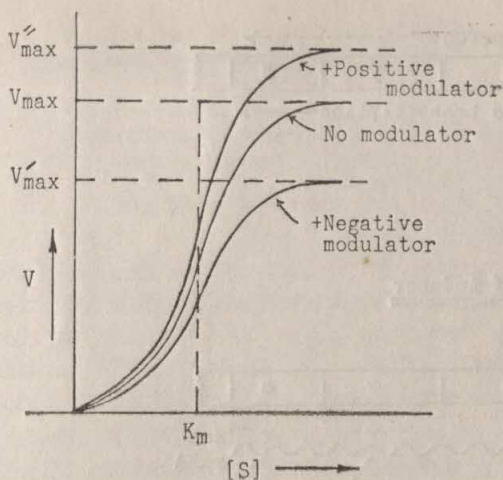


Fig. 5.14. Substrate saturation curves of a series of allosteric enzymes.

Sometimes an allosteric effector oppositely influences two allosteric enzymes catalyzing opposite reactions. For example, AMP allosterically activates phosphofructokinase for changing fructose 6-phosphate to FDP for glycolysis, and allosterically inhibits FDPase which hydrolyzes FDP to fructose 6-phosphate for gluconeogenesis.

5.4 REGULATION OF ENZYME SYNTHESIS

Induction and repression

Induction is the enhancement in enzyme synthesis through the promotion of gene transcription of the mRNA coding for that enzyme. **Repression** is the reduction or cessation in enzyme synthesis through a depression in gene transcription of the relevant mRNA. Some regulated enzymes, particularly of catabolic pathways, are *inducible enzymes*. Their synthesis ordinarily remains repressed, but is started or enhanced in presence of specific biomolecules called *inducers*. The inducer may be either the substrate or a *gratuitous inducer*, different from but structurally analogous to the substrate. Some other regulated enzymes, particularly of synthetic pathways, are *repressible enzymes*.

Ordinarily they are synthesized continuously but are repressed by specific metabolites. A repressible enzyme is frequently repressed by a metabolic product of the pathway using that enzyme (*feed-back repression*). Sometimes an enzyme of a multibranched metabolic pathway is repressed only in presence of the products of more than one branch of that pathway (*multivalent repression*).

Induction and repression are explained in prokaryotes by the *operon concept* of Jacob and Monod. An *operon* is defined as a segment of a DNA strand, consisting of a cluster of several *structural genes* and an *operator gene* adjacent to them. Each structural gene carries the genetic message for the synthesis of a specific protein or enzyme. The operator gene controls all the structural genes of the operon. RNA polymerase binds near the 3' end of the operator gene and moves in a 3'→5' direction along the structural genes, transcribing a single mRNA. The latter codes for the translation of several enzymes or proteins, each specified by one structural gene. Such an mRNA, coding for more than one enzyme or protein, is called a *polycistronic mRNA*. A *regulator gene*, located frequently at a distance from the operator gene on the same DNA strand, may transcribe an mRNA which helps to translate a *repressor* protein. The latter, when bound to the operator gene, blocks the movement of RNA polymerase along the operon and consequently represses all the enzymes specified by that operon.

The *lac operon* (Fig. 5.15) is an inducible catabolic operon of *E. coli*. It carries three structural genes, viz., *z*, *y* and *a* genes coding for β -galactosidase, galactoside permease and thiogalactoside transacetylase respectively. The first two enzymes participate in lactose catabolism while the last one is of uncertain physiological significance. The operator or *o* gene is located immediately on the 3' side of the *z* gene. RNA polymerase binds to a promoter or *p* site at the 3' side of the *o* gene.

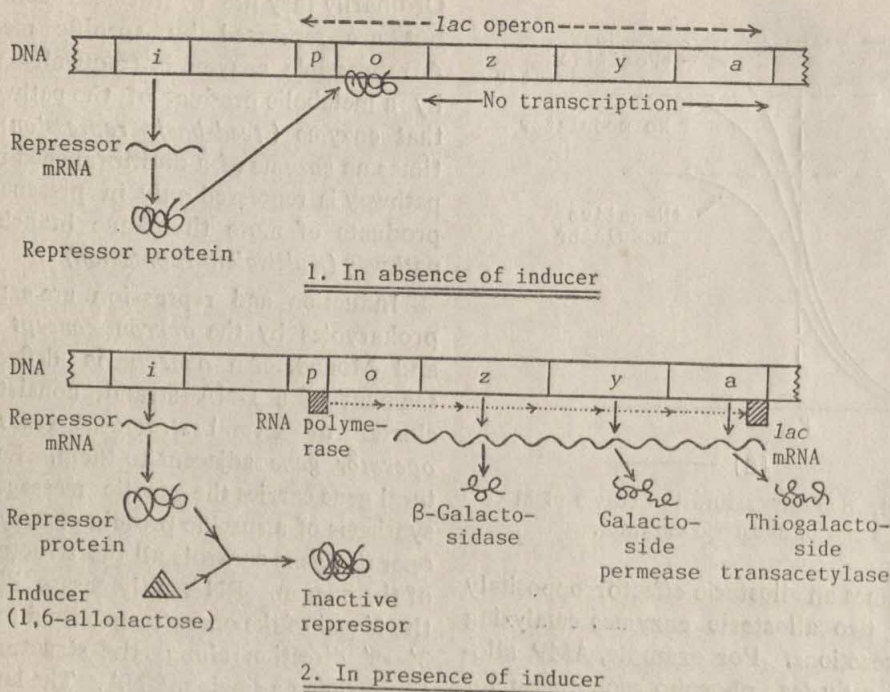


Fig. 5.15. Repression and induction of *lac* operon.

A regulator or *i* gene is located on the same DNA strand at a distance from the 3' end of the *o* gene and transcribes an mRNA. The latter codes for the translation of a tetrameric repressor protein. For inducible operons, the repressor is active by itself; thus, the *lac* repressor binds specifically with the *o* gene of *lac* operon, probably by diffusion along the DNA molecule itself, and blocks the flow of RNA polymerase across the *o* gene to the structural genes. The transcription of mRNA from all the structural genes remains consequently suspended and all three enzymes remain repressed. When lactose is present, small amounts of it are transglycosylated to 1,6-allolactose. The latter acts as the physiological inducer for *lac* operon and binds with both free and operator-bound repressor molecules to stop the operator-repressor interaction. The RNA polymerase can now bind to the *p* site, cross the free operator and

move along the structural genes, transcribing a polycistronic mRNA. The latter codes for translating all the three enzymes. Such an induction of a number of enzymes by an inducer influencing a single operator is called *co-ordinate induction*. β -Methylgalactoside and isopropylthiogalactoside act as gratuitous inducers for the *lac* operon.

The *ara* operon (Fig. 5.16) is another inducible catabolic operon of *E. coli*. It consists of an operator *araO* gene, a promoter *araI* site and three structural genes, viz., *araB*, *araA* and *araD* genes. The structural genes code for three arabinose catabolizing enzymes. A regulator or *araC* gene is located on the other side of *araO* and codes for a repressor protein. In the absence of arabinose, the repressor binds to *araO* gene and represses all three enzymes by blocking the RNA polymerase-mediated transcription of a polycistronic mRNA by the structural genes.

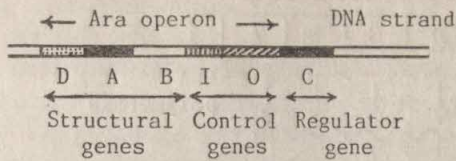


Fig. 5.16. Ara operon.

Arabinose acts as the inducer. It changes the conformation of the repressor which then binds to the *araI* site as an *activator* to enhance RNA polymerase activity. This induces the synthesis of the relevant enzymes.

Cyclic AMP mediates in activating many inducible catabolic operons like *lac* and *ara* operons of *E. coli*. cAMP binds with a dimeric *catabolite gene activator protein* (CAP) to form a CAP-cAMP complex. Unlike free CAP, this complex binds to the promoter site immediately beside the binding site for RNA polymerase. This probably provides an additional site for RNA polymerase-DNA interaction and enables the polymerase to commence transcription of the structural genes. Glucose reduces intracellular cAMP concentration and thereby leads to a repression of catabolic operons in *E. coli* (*catabolite repression*).

The *trp operon* (Fig. 5.17) is a repressible synthetic operon of *E. coli*. It carries five structural genes, viz., A, B, C, D and E genes, coding for five enzymes for synthesizing tryptophan from chorismate. The operon also has an operator O gene overlapping with a promoter site. A regulator or R gene is located at a considerable distance from the O gene. The R gene transcribes an mRNA which translates an inactive *aporepressor*, unable to bind alone to the operator. Tryptophan acts as a *corepressor* and binds with the aporepressor to form an active *holorepressor*. The latter binds to the operator gene to block the binding of RNA polymerase with the promoter site. Transcription of the structural genes is thus suspended, causing a repression of all the five enzymes.

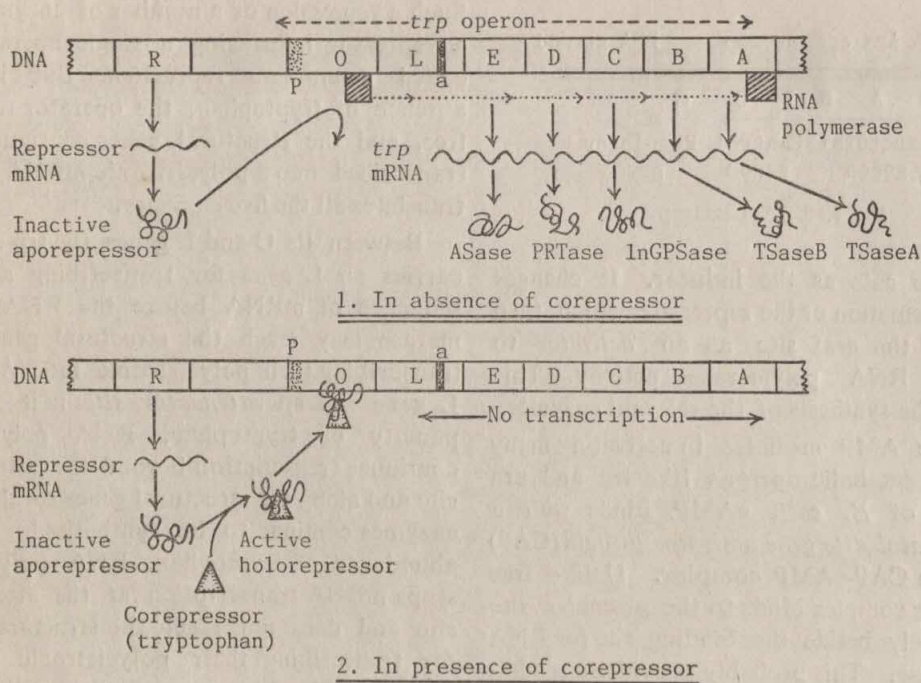
Such a repression of a number of enzymes by a repressor influencing a single operator is called *co-ordinate repression*. But in the absence of tryptophan, the operator remains free, and the structural genes continue to be transcribed into a polycistronic mRNA which translates all the five enzymes.

Between its O and E genes, the *trp operon* carries an L gene for transcribing a leader sequence of mRNA before the RNA polymerase may reach the structural genes for transcribing their polycistronic mRNA. The L gene has an *attenuator site* in it. In the paucity of tryptophan, RNA polymerase continues transcription beyond the attenuator site and along the structural genes so that the enzymes continue to be synthesized. But in abundance of tryptophan, RNA polymerase stops mRNA transcription at the attenuator site and does not reach the structural genes for transcribing their polycistronic mRNA (*attenuation*). This suspends the synthesis of the enzymes.

Some enzymes are synthesized continuously without any repression and need no inducer. They are called *constitutive enzymes*. Constitutivity may result from the mutation of either the regulator or the operator gene. In both cases, the repressor produced by the regulator fails to bind with and repress the operator. The structural gene consequently continues an uninterrupted synthesis of the enzyme.

Eukaryotic regulations

The concept of operon as a cluster of structural genes cannot be extended to eukaryotes, because they possess *monocistronic mRNA*, each coding for a single peptide. However, many catabolic enzymes like threonine dehydratase and tryptophan 2,3-dioxygenase appear to be inducible enzymes. Steroid hormones like cortisol and active vitamin D derivatives seem to influence specific eukaryotic genes to induce the synthesis of

Fig. 5.17. Repression of *trp* operon.

many enzymes; e.g., the induction of FDPase and PEP carboxykinase by cortisol.

In eukaryotes, enzyme synthesis may also be regulated by (a) repetitive replication of some genes, causing an amplification of such genes, (b) rearrangement of genes in DNA strand, (c) control of RNA transcription by enhancer sequences in DNA and steroid-induced changes in genes, (d) differential processing of mRNA after completion of their transcription, (e) differential translation of mRNA, and (f) steroid-induced changes in the stability of mRNA.

5.5 ENZYMES FOR RATE-LIMITING STEPS

The rate-limiting or committed step of a metabolic pathway is that reaction which determines the rate and direction of the entire pathway. It should fulfil following criteria. (a) Of all the enzymes of the pathway, the enzyme for the rate-limiting step should have the lowest substrate affinity and the highest

K_m . Thus, the velocity of the reaction catalyzed by it will be the lowest when saturating

TABLE 5.7. Rate-limiting enzymes of some metabolic pathways.

Pathways	Rate-limiting enzymes
Adipose tissue lipolysis	Hormone-sensitive lipase
Cholesterol synthesis	HMG-CoA reductase
Citric acid cycle	α -Ketoglutarate dehydrogenase
Fatty acid synthesis	Acetyl-CoA carboxylase
Gluconeogenesis	FDPase, PEP carboxykinase
Glycogenesis	Glycogen synthase
Glycogenolysis	Glycogen phosphorylase
Glycolysis	Phosphofructokinase
Pentose phosphate pathway	Glucose 6-phosphate dehydrogenase
Purine synthesis	PP-ribose-P glutamyl amidotransferase
Urea formation	Carbamoyl phosphate synthase

substrate concentrations are provided for all the enzymes. So, the rate of this step will determine the rate of the entire series of reactions. The over-all rate may be changed by altering the activity of the rate-limiting enzyme. (b) The rate-limiting enzyme should be a *regulated enzyme*. Its activity should be controlled by allosteric modulations or reversible covalent modifications and its synthesis regulated by induction and repression. (c) The rate-limiting step is *practically unidirectional* or irreversible under the conditions *in vivo* and determines the direction of the entire pathway. (d) The step is usually an *initial step* of a pathway so that the intermediates of earlier steps should not accumulate in case of the feed-back inhibition of the rate-limiting enzyme.

5.6 CLASSIFICATION OF ENZYMES

Enzymes are broadly classified into six classes according to the types of reaction catalyzed by them. Each class is further subdivided into many sub-classes and each sub-class into several sub-sub-classes (Table 5.8). Every enzyme has been given an *enzyme code number* (EC) in four digits standing respectively for class, sub-class, sub-sub-class and serial number of the particular enzyme.

1. *Oxidoreductases*: Enzymes of this class catalyze oxidations and reductions of their substrates. Oxidations are carried out either by addition of oxygen to the substrate or by the removal of electron (and H^+) from the substrate. This class is subdivided according to the electron donor or acceptor group of the substrate.

2. *Transferases*: These enzymes transfer a particular group from one substrate to another. Transferases are divided into sub-classes according to the groups transferred.

3. *Hydrolases*: These enzymes cleave their substrates by the hydrolysis of a covalent bond. They are divided into sub-classes according to the bonds hydrolyzed.

4. *Lyases*: They cleave a covalent bond in the substrate to change it to more than one product, but the cleavage does not involve any hydrolysis. Their action frequently produces a double-bond in one of the products. Lyases are classified according to the atoms connected by the bond which is broken.

5. *Isomerases*: They change their substrates to their isomers by intramolecular rearrangements and are classified according to the type of isomerization.

6. *Ligases*: These enzymes bind two substrate molecules together, by forming bonds with the energy from the simultaneous breakdown of high-energy compounds like ATP. Ligases are subdivided according to the atoms connected by the bond formed.

5.7 ENZYMES IN CLINICAL DIAGNOSIS

The enzymes of a diseased or damaged tissue may leak out into the blood, when the permeability of the affected tissue is greatly enhanced. So, the activity of an enzyme, present in high concentration in the affected tissue, may rise significantly in the serum. Changes in serum enzyme activities help in the diagnosis of diseases, depending on (a) the enzymes or specific isozymes present in higher concentration in the affected tissue or organ than in other organs, (b) the severity of damage of the tissue, and (c) the stage of maximum permeability and its duration.

For measuring an enzyme activity, a measured amount of serum, blood or any other test material containing the enzyme is mixed with cofactors, buffers and specific concentrations of its substrate, and is incubated for a specific period; finally, either the residual amount of the unchanged substrate, or the amount of the product, or the enzyme-induced change in properties like optical density is estimated. Different isozymes of an enzyme are usually separated by electrophoresis or ion exchange chromatography, or

TABLE 5.8. Examples of enzymes and their classes.

EC No.	Enzyme	Source	Substrates	Products	Sub-class
<i>Class : 1. Oxidoreductases</i>					
1.1.1.27	Lactate dehydrogenase	Heart, muscle, liver	Lactate	Pyruvate	Electron-donor CH(OH) group
1.2.3.2	Xanthine oxidase	Liver	Xanthine	Uric acid	Electron-donor aldehyde or ketone group
1.4.3.3	D-Amino acid oxidase	Liver, kidney	D-Amino acids	Imino acids	Electron-donor CH (NH ₂) group
1.9.3.1	Cytochrome oxidase	Mitochondria	Reduced cytochrome C	Oxidized cytochrome C	Electron-donor heme group
<i>Class : 2. Transferases</i>					
2.1.1.2	Guanidoacetate methyltransferase	Liver	Guanidoacetate + S-adenosylmethionine	Creatine + S-adenosylhomocysteine	One-carbon transferases
2.3.1.6	Choline acetylase	Motor cortex, caudate nucleus	Acetyl-CoA + choline	CoA + acetylcholine	Acyltransferases
2.6.1.2	Glutamate pyruvate transaminase	Liver, serum	L-Glutamate + pyruvate	α -Ketoglutarate + L-alanine	Nitrogenous group transferases
2.7.1.2	Glucokinase	Liver	ATP + D-glucose	ADP + D-glucose 6-phosphate	Phosphotransferases
<i>Class : 3. Hydrolases</i>					
3.1.3.9	Glucose 6-phosphatase	Liver, kidney	D-Glucose 6-phosphate	D-Glucose + Pi	Esterases
3.2.1.1	α -Amylase	Saliva, pancreatic juice	Starch	Dextrins	Glycoside hydrolases
3.4.4.1	Pepsin	Gastric juice	Proteins	Peptones	Peptide hydrolases
<i>Class : 4. Lyases</i>					
4.1.1.22	Histidine decarboxylase	Mast cells	L-Histidine	Histamine + CO ₂	C-C lyases
4.2.1.2	Fumarase	Heart	L-Malate	Fumarate + H ₂ O	C-O lyases
4.3.2.1	Argininosuccinase	Liver, kidney	L-Argininosuccinate	L-Arginine + fumarate	C-N lyases
<i>Class : 5. Isomerases</i>					
5.1.3.2	UDP-glucose epimerase	Liver	UDP-glucose	UDP-galactose	Epimerases & racemases
5.2.1.3	Retinene isomerase	Retina, liver	All- <i>trans</i> -retinene	11- <i>cis</i> -Retinene	<i>cis-trans</i> isomerases
5.3.1.1	Triose phosphate isomerase	Muscle	Glyceraldehyde 3-phosphate	Dihydroxyacetone phosphate	Aldose-ketose isomerases
<i>Class : 6. Ligases</i>					
6.1.1.7	Alanyl-tRNA synthase	Yeast	L-Alanine + tRNA	L-Alanyl-tRNA	C-O ligases
6.2.1.1	Acetate thiokinase	Liver	Acetate + CoA	Acetyl-CoA	C-S ligases
6.3.1.2	Glutamine synthetase	Kidney	L-Glutamate + NH ₃	L-Glutamine	C-N ligases
6.4.1.1	Pyruvate carboxylase	Liver, kidney	Pyruvate + CO ₂	Oxaloacetate	C-C ligases

are differentiated by thermal inactivation and inhibition by specific inhibitors.

1. Serum alkaline phosphatase : Alkaline phosphatases are rich in bones, small intestine, liver, kidneys and placenta. They hydrolyze organic phosphate esters to liberate inorganic phosphate (Pi) at the serum pH of 7.4 although their optimum pHs lie around 9. The total activity of the isozymes in the normal serum amounts to 1.5-4 Bodansky units per 100 ml. Each Bodansky unit indicates the liberation of 1 mg Pi per 100 ml serum on its incubation with β -glycerophosphate for 1 hour at 37°C and pH 8.6. High calcification in the growing bones is reflected in high alkaline phosphatase activity—5-12 Bodansky units per 100 ml of the child serum. In rickets, osteomalacia, hyperparathyroidism and osteogenic sarcoma, the serum alkaline phosphatase activity rises even by 10-20 times due to the *thermolabile phenylalanine-resistant isozyme* from bones; the low activity in hypophosphatasia correlates with reduced calcification of bones. In liver diseases like obstructive jaundice and acute viral hepatitis, serum alkaline phosphatase activity rises due to an increased output of the *thermostable phenylalanine-resistant isozyme* from the liver.

2. Serum acid phosphatase : Acid phosphatase isozymes hydrolyze organic phosphates to liberate Pi at the serum pH although their optimum pHs lie around 5. Normal serum contains only small amounts of acid phosphatases (0-1.1 Shinwara units per 100 ml). Each unit corresponds to 1 mg Pi liberated from β -glycerophosphate on incubation with the serum for 1 hour at pH 5.0. Serum isozymes come normally from bones, liver, kidneys, pancreas and spleen, and are not inactivated by tartrate or ethanol although some are inactivated by formaldehyde. The adult prostate and prostatic fluid are rich in a *tartrate-labile, ethanol-labile but formaldehyde-resistant isozyme*; this isozyme increases in the serum up to 10 times in

prostatic carcinoma with metastasis, thus raising the total serum acid phosphatase activity, while the *ethanol-resistant isozymes* continue to remain low. In bone diseases like osteitis deformans and in breast carcinoma, serum acid phosphatase activity rises due to the rise in *ethanol-stable isozymes* and *formol-stable isozymes* respectively, but the tartrate-labile isozyme is almost unchanged.

3. Serum lactate dehydrogenase : Lactate dehydrogenase (LDH) oxidizes lactic acid to pyruvic acid, using NAD⁺ as the coenzyme. It can also catalyze the reverse reaction. Of the five isozymes, LDH-I₁ predominates in the cardiac muscle, LDH-I₅ in the liver and skeletal muscles, LDH-I₂ and LDH-I₃ in the brain, and LDH-I₂ and LDH-I₁ in kidneys and RBC. The total serum activity of the five LDH isozymes amounts normally to 200-680 units per ml of serum. The unit is a spectrophotometric measure of the decrease in optical density of the test solution in 5 minutes due to the oxidation of NADH to NAD⁺ during the enzyme-catalyzed reduction of 0.1M sodium pyruvate. Serum LDH-I₁ rises in myocardial infarction. Serum LDH-I₅ increases in liver diseases and progressive muscular dystrophies.

4. Serum α -hydroxybutyrate dehydrogenase : This enzyme (HBD) reduces α -ketobutyric acid to α -hydroxybutyric acid, using NADH as the coenzyme. It also catalyzes the reverse reaction. Cardiac muscle is the main source of the serum HBD. Serum HBD activity normally ranges from 55 to 125 IU per litre. The unit is a spectrophotometric measure of the decrease in extinction of the test solution per minute due to the oxidation of NADH to NAD⁺ during the enzyme-catalyzed reduction of 0.1M sodium α -ketobutyrate. Serum HBD rises in myocardial infarction, but shows no change in hepatitis, obstructive jaundice and other hepatic diseases.

5. Serum creatine phosphokinase : This enzyme (CPK) phosphorylates creatine to creatine phosphate, utilizing ATP as the phosphate

donor. CPK₁ (BB), CPK₂ (MB) and CPK₃ (MM) are its dimeric isozyme forms. CPK isozymes occur mainly in cardiac and skeletal muscles and brain. So, plasma CPK activity rises and the CPK isozyme pattern changes significantly in myocardial infarctions and in traumas and dystrophies of skeletal muscles. In myocardial infarctions, plasma CPK₂ activity may reach even twenty times its normal value and may contribute 5-20% of the total plasma CPK activity in contrast to its maximum normal 2% contribution.

6. Serum glutamate oxaloacetate transaminase: This enzyme (SGOT) transfers an amino group from glutamic acid to oxaloacetic acid and also catalyzes the reverse reaction. SGOT activity amounts to 8-40 units per ml of normal serum. The unit is a measure of the decrease in optical density of the test solution per minute due to the enzyme-catalyzed reaction. Heart, liver and skeletal muscles are rich in the enzyme in a descending order. SGOT rises even by 5-30 times for a short period during and following myocardial infarctions even when ECG gives no clear indication. The cytoplasmic isozyme of SGOT predominates in the serum in less severe myocardial lesions, but the mitochondrial isozyme also rises in severe myocardial necrosis. SGOT far exceeds SGPT in myocardial infarctions. SGOT also rises in congestive cardiac failure, acute pericarditis and infective hepatitis, but usually lags behind SGPT in hepatic disorders. Small rises in SGOT occur in neuromuscular dystrophies.

7. Serum glutamate pyruvate transaminase: This enzyme (SGPT) transfers an amino group from glutamic acid to pyruvic acid and also catalyzes the reverse reaction. SGPT activity amounts to 9-30 units per ml of normal serum. The unit measures a specific decrease in the optical density of the test solution due to the enzyme-catalyzed reaction. Liver has the highest amount of this enzyme. Cardiac muscles are poorer. In infective and toxic hepatitis, homologous serum jaundice

and some forms of obstructive jaundice, serum SGPT rises rapidly, exceeding the SGOT activity. But in myocardial infarctions, SGPT lags far behind SGOT.

8. Serum cholinesterase: *Pseudocholinesterase* of serum comes mainly from the liver and hydrolyzes acetylcholine, succinylcholine, butyrylcholine and benzoylcholine at almost similar speeds. Serum pseudocholinesterase activity is reduced in hepatic disorders, cachexia, cancer, uremia, tuberculosis, and treatment with muscle relaxants like succinylcholine chloride. *True cholinesterase* of brain and erythrocytes hydrolyzes acetylcholine far more rapidly than other choline esters. Both true and pseudo cholinesterases fall in blood in poisoning with carbamate and organophosphate pesticides.

9. Serum amylase: This starch-hydrolyzing enzyme enters the blood from the pancreas. Its normal activity is 60-180 *Somogyi units* per 100 ml serum. Each unit corresponds to the liberation of 1 mg of glucose from starch on its incubation with the serum for $\frac{1}{2}$ hour at 37°C. Serum amylase activity normally ranges between 3 and 10 *Wohlgemuth units* per ml of serum. Each such unit corresponds to the hydrolysis of 1 mg of starch on incubation with the serum for 30 minutes at 37°C. Acute pancreatitis, necrosis of pancreatic acini, or obstruction of pancreatic outlets raises the serum amylase activity, but the rise subsides in a short time.

10. Serum lipase: This esterase enters the blood from the pancreas and has a normal activity of 0.1-1.5 units per ml of serum. Each unit corresponds to 1 ml of N/20 NaOH used in neutralizing the fatty acids liberated by the hydrolysis of olive oil on its incubation with serum at 37°C for 24 hours. Serum lipase activity increases in acute pancreatitis, necrosis of pancreatic acini, obstruction of pancreatic outlets, and often in chronic pancreatitis also.

6. WATER AND MINERALS

Human body contains about 60-70% water and about 8.5% minerals. Minerals include relatively larger amounts of sodium, potassium, calcium, magnesium, phosphorus, chlorine and sulfur, and very small amounts of iron, copper, cobalt, manganese, zinc, selenium, iodine, fluorine, chromium, cadmium and molybdenum.

6.1 WATER METABOLISM

Sources : Body water comes from three main sources.

1. *Food water :* About 700-1000 ml of water come from foods every day.

2. *Water in drinks :* Man must get at least 600 ml of water from drinks every day to enable the kidneys to eliminate nitrogenous waste products. To serve as sources of water, beverages must be diluter than the maximum concentration of urine.

3. *Metabolic water :* About 300-400 ml of water are daily produced by oxidation. Each gram of carbohydrate, fat and protein yields 0.55, 1.06 and 0.45 g of water respectively on complete oxidation. Kangaroo rats get 90% of their entire water requirement from metabolic water alone, because very little water is lost in their sweat, respiratory air, feces and urine. But in other mammals like man, donkey and camel, the total water requirement cannot be supplied by metabolic water alone ; they must drink some water.

Losses : Water is eliminated through several channels.

1. *Insensible losses :* About 1000-1200 ml of water may be unconsciously lost daily from the human body. These *obligatory losses* take place irrespective of the availability of water.

(a) *Insensible cutaneous evaporation :* Man loses about 600 ml of water daily by cutaneous diffusion and evaporation, exclusive of sweating. This *insensible perspiration* decreases non-linearly with the rise in the vapour pressure of air.

(b) *Pulmonary evaporation :* Normally about 400 ml of water are evaporated from pulmonary alveoli and respiratory passages and eliminated with expired air every day.

(i) If the inspired air is *humid*, pulmonary evaporation of small amounts of water saturates it and evaporative loss falls. Inhalation of dry air increases pulmonary evaporation. (ii) Rise in the *respiratory rate* increases evaporation. (iii) Rise of *body temperature* increases the evaporative loss by enhancing the respiratory rate. It also warms up the inspired air to lower its relative humidity, and allows more water vapour to be added by pulmonary evaporation. (iv) *Exercise and high metabolic rate* increase the respiratory loss by raising the respiratory rate and the body temperature.

2. *Loss of sweat :* Loss of water as visible sweat amounts to 0-12 litres day⁻¹ in man. Temperature and exercise increase this loss. Man, other primates, horse, donkey and mule may lose large volumes of sweat. Cattle, sheep, goat, camel, cat and dog have fewer sweat glands and lose comparatively less water in sweat. Nonmammals, rodents and marine mammals do not possess sweat glands and lose no water in sweat. Camels secrete sweat only when the body temperature exceeds 40.5°C ; this higher thermal tolerance considerably reduces the loss of water in sweat.

3. *Fecal loss :* Normally, about 100-200 ml may be *obligatorily* lost with feces due to

osmotic effects of fecal constituents. Diarrhoea increases the fecal loss.

4. *Renal loss* ; About 14 litres of fluid are filtered into kidney tubules in 24 hours. But about 99% of the water is reabsorbed leaving only 1000-1500 ml of urine. Of this, nearly 600 ml must be excreted for eliminating the urinary solutes (*obligatory water excretion*). Remaining urinary water is not essential for elimination of solutes and depends largely on the availability of water (*facultative water excretion*). The capacity for renal reabsorption is limited—the maximum specific gravity of human urine is about 1.040. With sufficient water available, the facultative loss increases and urine gets more hypotonic due to reduced renal reabsorption of water. Restricted intake or increased loss of water through sweat, feces or respiration minimizes the facultative loss, making the urine highly hypertonic.

The total daily loss of water is ordinarily about 2500 ml in adults—about 700 ml as facultative loss in urine and about 1800 ml as obligatory loss in urine, feces, expired air and insensible perspiration.

Requirements : About 2200 ml of water must come in foods and drinks every day. This, along with about 300 ml of metabolic water, will replace the amount lost.

Biological roles :

1. *Solvent action* : Water is the *most abundant* of molecules in the living body and serves as the solvent both around and inside the cells. Both extracellular and intracellular fluids are thus aqueous solutions. In these aqueous media, water molecules occur as short-lived lattice-like aggregates of 3-4 molecules each, due to hydrogen bonds joining the hydrogen nuclei of one molecule and the oxygens of two other molecules (Fig. 6.1). This depends on the *polar nature* of water molecules with more electropositive hydrogen nuclei at one pole and more electronegative

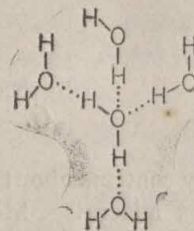


Fig. 6.1. Hydrogen bonding of water molecules.

oxygen at the other pole of the molecule. However, water remains fluid in spite of such hydrogen-bonded aggregates because the hydrogen bonds are transient and have a very rapid rate of dissociation and regeneration. Polar groups of solutes get hydrogen-bonded with polar molecules of water and are consequently hydrophilic (water-attracting) in nature. Such hydrogen bonding joins the polar solutes to the lattice structure of water and thus keeps them in solution. This solvent action of water is further accentuated by the high *dielectric constant* of water, which signifies its high capacity to reduce the electrostatic attraction between counterionic groups of dissolved solutes, preventing their aggregation and keeping them dispersed in solution. The dissolved polar solutes can thus diffuse uniformly in aqueous body fluids and are easily transported therein. Nonpolar molecules cannot join the lattice structure of liquid water and cannot dissolve in it because their nonpolar groups cannot form hydrogen bonds with water. This forces them either to remain associated with polar molecules like plasma proteins, or to squeeze out intervening water molecules and arrange themselves in special structural aggregates like micelles and bilayers with minimum contact between their nonpolar groups and water.

2. *Molecular conformations and structural organizations in aqueous media* : Molecular conformations and structural organizations of biomolecules depend largely on the water of biological fluids. Biological activities of

proteins and nucleic acids depend on their specific three-dimensional conformations in aqueous media, which they attain by spontaneous folding and coiling of the molecule to expose many of their polar groups on their water-adjointing surfaces and to hide most nonpolar groups in the nonaqueous interior of the coils. All biological membranes consist of lipid bilayers resulting from specific arrangements and orientations of amphipathic lipid molecules in aqueous media.

3. *Fluid exchange*: Water is one of the smallest of biomolecules. Its *small molecular size* and polar nature enable its molecules to pass freely through the hydrophilic pores of most biological membranes. This allows a constant exchange of water between the cell and the extracellular fluid as also between different intracellular compartments. Small molecules of water can also pass into narrow niches and crevices of three-dimensional macromolecules, causing their *hydration*.

4. *Ionizations*: Water itself ionizes into into H^+ and OH^- to provide a specific H^+ concentration in the biological fluids, depending on temperature. Ionization of mineral salts in aqueous body fluids gives mineral ions like Na^+ , K^+ and Cl^- , providing for electrical conductivity of body fluids and membrane polarization and depolarization. Proteins, nucleic acids, phospholipids and mucopolysaccharides accept or donate H^+ from or to water of body fluids to exist in specific ionic states necessary for their biological activities.

5. *Reaction medium and reactant*: Water acts as a solvent for many biomolecules and consequently forms the medium where they may intermix and interact easily. Water donates H^+ and OH^- for many biochemical reactions. It is a reactant in hydrolysis and hydration. During photosynthesis, its OH^- acts as the donor of the electron to chlorophyll in replacement of the electron ejected from chlorophyll on absorbing light photons

and gets oxidized to O_2 while the H^+ of water reduces $NADP^+$ to $NADPH$.

6. *Homeostasis*: Homeostasis or constancy of internal environment is maintained largely with the help of body fluid water. For example, temperature homeostasis is maintained by uniform distribution of body heat and by cutaneous heat loss through convection and radiation, all dependent on high specific heat and mobility of water in blood vessels; heat loss also involves cutaneous evaporative loss of heat by evaporation of sweat water.

Water balance: An animal is in *water equilibrium* when the total loss of water is exactly replaced by the amount obtained from various sources. When more water is eliminated from the body than what is taken in, the body is in *negative water balance*, e.g., in water deprivation, diarrhoea, violent vomiting and excessive sweating. If the loss of water is less than the amount obtained from different sources, the body is in *positive water balance*, e.g., during growth and pregnancy.

Distribution: To estimate the *total body water* (36-42 litres in adult man), known amounts of deuterium oxide or antipyrine are intravenously injected. These substances easily enter all body fluids and cells. The dilution of the injected substance is subsequently determined.

Of the total body water, 55% (20-23 litres) exists as *intracellular water* while 45% (14-18 litres) is distributed as *extracellular water*.

Extracellular fluids (ECF): To estimate the ECF volume, a substance which passes out easily from blood vessels but enters less easily into cells, e.g., inulin, thiosulfate, thiocyanate, mannitol or radioactive ^{24}Na , is injected intravenously and its dilution is subsequently determined. Deducting this ECF volume from the total body water volume, the *intracellular water volume* is also estimated. ECF exists in several compartments:

(a) *Blood plasma*: This amounts to 2.5-3.5 litres or 8% of the total body water.

Its volume is estimated by intravenously injecting a substance that cannot pass out from the vessels easily, e.g., Evans' blue (T-1824), ^{131}I -albumin or ^{32}P -erythrocytes, and determining its dilution in the plasma.

(b) *Interstitial fluid and lymph*: They occur in extravascular intercellular spaces and lymph vessels, respectively. They amount to 7-8 litres or 20% of the body water.

(c) *Special extracellular fluids*: These amount to 6.5-7.5 litres or 17-18% of total body water and include the following fluids:

(i) *Transcellular fluids* include aqueous humor, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, fluids in glands and mucous membranes, etc.; they amount to 800-1200 ml or 2-3% of the body water.
 (ii) *Extracellular fluids in dense connective tissues* like cartilages and bones amount to 5.5-6.5 litres or 15% of the body water and remain isolated from other extracellular compartments because of very slow fluid exchange with other extracellular fluids. The ECF volumes, determined by inulin, mannitol or thiosulfate, are generally exclusive of the fluid in dense connective tissues; but the volumes determined by thiocyanate or ^{24}Na are inclusive of this fluid and a deduction of the plasma volume from such ECF volumes gives the sum of interstitial-lymph volume and specialized extracellular fluid volume.

Regulation of water metabolism:

1. *Vasopressin or antidiuretic hormone*: It enhances the facultative reabsorption in kidneys by increasing the permeability of distal tubules and collecting ducts to water.

2. *Hypothalamus*: Dehydration causes osmoconcentration of plasma; this stimulates a *thirst centre* in the hypothalamus, producing thirst and provoking the animal to drink the required amount of water. Osmoconcentration of plasma also stimulates *supraoptic and paraventricular nuclei* of hypothalamus; nerve impulses from them increase the release of

vasopressin from neurohypophysis into the blood. Lesions in the supraoptico-paraventricular region or in the neurohypophysis produce *diabetes insipidus* characterized by large volumes of hypotonic urine.

3. *Solutes*: Osmotic effect of Na^+ helps to retain water in extracellular fluids. Rise in plasma Na^+ raises the ECF volume in primary aldosteronism while an increase in urinary Na^+ raises the urinary water output in Addison's disease. K^+ helps to retain water in the cell. Plasma proteins help to retain water in blood by their osmotic effects. Rise in urinary urea or excretion of glucose in urine increases osmotically the urinary loss of water.

4. *Aldosterone*: Osmoconcentration, low plasma Na^+ or a fall in plasma volume stimulates aldosterone secretion; this cortical steroid then increases Na^+ reabsorption from urine and consequently increases the reabsorption of water. By maintaining the normal $\text{Na}^+:\text{K}^+$ balance in different body fluid compartments, aldosterone maintains a normal distribution of water in extracellular and intracellular fluids.

5. *Renin-angiotensin system*: A fall in circulating blood volume stimulates renin secretion from kidneys. Renin promotes angiotensin formation in plasma. Angiotensin II stimulates the synthesis and secretion of aldosterone and the release of vasopressin, and thereby enhances renal reabsorption of Na^+ and water.

6. *Prostaglandins*: They may maintain glomerular filtration in spite of hypotension, by causing renal vasodilatation. They may also enhance the urinary loss of water by inhibiting the antidiuretic effect of vasopressin and by increasing the urinary Na^+ .

Dehydration: An uncompensated loss of water from the body or a severe restriction of water intake may cause dehydration.

(a) *Pure water depletion*, without salt depletion, results from insufficient water intake

due to failure of swallowing or acute water scarcity. This type of dehydration raises the concentration and osmotic pressure of ECF with a consequent outflow of the intracellular water to the ECF. The ECF volume is thus largely restored, but cells suffer from osmotic concentration. Symptoms include poor salivation, dry shrunken skin, nausea, reduced sweating and intense thirst. Hypertonicity of blood lowers the urine volume and concentrates the urine, reducing the urinary elimination of NPN and acids; the consequent acidosis may lead to coma. Enhanced catabolism of tissue proteins and lipids produces more metabolic water, but aggravates the dry shrunken condition of skin and the elevation of blood NPN and affects neuromuscular tissues, leading to exhaustion, incoordination and confusion. Salt concentration rises in plasma and significant amounts of Cl^- continue to be excreted in the urine. Death occurs in man due to renal failure, acidosis, circulatory collapse, intracellular hyperosmolality or neural depression, when body water falls by 20%; camel, donkey and sheep succumb only when 30% of the body water has been lost.

Injection or drinking of concentrated salines like sea-water, or failure of Na^+ excretion (e.g., in Cushing's syndrome and primary aldosteronism) may cause hypertonicity of ECF, consequent withdrawal of water from tissue cells, dehydration of tissues, but a rise in ECF volume. Mg^{2+} of sea-water may also increase intestinal loss of water due to its osmotic effects in the intestinal lumen.

(b) *Salt depletion* may produce a dehydration of ECF but a rise in intracellular fluid volume in Addison's disease, diabetic polyuria, severe diabetes or vomiting, if the excessive loss of NaCl and water is sought to be compensated by taking enough water without any salt. The osmotic pressure of ECF and the plasma Na^+ and Cl^- may fall;

to compensate, Cl^- practically ceases to be eliminated in the urine. The relative hypertonicity of the intracellular fluid draws water osmotically from the ECF to the cells, causing a swelling of tissue cells (*intracellular edema*) and a fall in the intracellular osmotic concentration. This reduces the volume of ECF including plasma, reflected in an elevated hematocrit value. The fall in the plasma volume leads to a rise in body temperature, hypotension, circulatory failure and a consequent depression of renal function, leading to high blood NPN and acidosis. Severe thirst is, however, absent.

(c) *Simultaneous water-salt depletion*, followed by insufficient water intake, produces severe dehydration. As the water loss exceeds the salt loss, the resulting hypertonicity of ECF draws fluid from cells to ECF. The latter also continues to fall in volume due to urinary loss.

6.2 CALCIUM AND PHOSPHORUS METABOLISMS

Sources : Milk, cheese, khoa, hard water, lime used with betel, dark-green leaves, salmon, sardine, carrot and finger millet ('ragi') are important dietary sources of calcium. Sources of phosphorus include wheat, maize, oatmeal, egg, milk, cheese, salmon, chicken and mutton.

Requirements : The daily requirements for calcium are : adult man/woman : 500 mg ; pregnant or lactating woman : 1 g ; infants and children : 0.4-0.6 g, according to age. An almost identical amount of phosphorus is required.

Functions of calcium :

1. *Constituent of bones and teeth* : Calcium and phosphorus are the principal minerals of bones and teeth. They occur in the bone matrix and the enamel, dentin and cementum of teeth mainly as rod-shaped or platelet-shaped crystals of calcium hydroxyapatites like $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ and carbonate

apatites like $\text{Ca}_{2.2}(\text{PO}_4)_{1.4}\text{CO}_3$, and to some extent as amorphous deposits of calcium carbonates and phosphates. These minerals give hardness, strength and concrete-like elastic modulus to those tissues.

2. *Blood coagulation*: Ca^{2+} ions get chelated to the additional γ -carboxyl groups of numerous γ -carboxyglutamate residues of prothrombin. This helps to anchor prothrombin to the surface of phospholipid membranes from damaged or clumped platelets. It places prothrombin close to activated Stuart factor (X_a) and serum accelerin (V_a) to enhance many thousandfold the activation of prothrombin to thrombin by those factors. Thus, Ca^{2+} helps in blood coagulation. Precipitation of Ca^{2+} by oxalate or fluoride renders the blood noncoagulable.

3. *Enzyme action*: Ca^{2+} acts as either modulator or cofactor of many enzymes like adenylate cyclase, myosin light-chain kinase, and protein kinases (liver and muscle). In most cases, Ca^{2+} first binds with *calmodulin*, a small thermolabile anionic protein of cytosol and membranes, to form a Ca^{2+} -calmodulin complex. The latter exerts the modulating action on enzymes.

4. *Synaptic transmission*: Ca^{2+} of extracellular fluids helps in synaptic transmission by promoting the release of acetylcholine from the presynaptic neuron. As the nerve impulse reaches the axon terminal of the latter, the presynaptic membrane is depolarized; this opens up calcium channels in the presynaptic membrane, leading to an enhanced Ca^{2+} influx from the extracellular fluid into the presynaptic axon terminal. This Ca^{2+} influx induces the emigration and fusion of synaptic vesicles with the presynaptic membrane, and enhances the release of acetylcholine from those vesicles into the synaptic cleft separating the presynaptic and postsynaptic membranes.

5. *Muscle contraction*: Ca^{2+} is essential for both the actin-based regulation of skeletal and cardiac muscle contractions and the

myosin-based regulation of smooth muscle contraction.

(a) In resting *skeletal and cardiac muscle fibres*, the sarcoplasmic concentration of Ca^{2+} is maintained near 10^{-6}M by a Ca^{2+} pump mechanism of the sarcoplasmic reticular membrane. The Ca^{2+} pump consists of Ca^{2+} -dependent ATPase, an integral membrane protein which actively transfers two Ca^{2+} from the sarcoplasm to the sarcoplasmic reticular lumen with the energy of hydrolysis of one ATP bond. Inside the sarcoplasmic reticulum, another protein called *calsequestrin* binds Ca^{2+} to keep it sequestered in the sarcoplasmic reticulum. In this way, Ca^{2+} concentration is maintained near 10^{-2}M inside the sarcoplasmic reticulum in resting muscles. Stimulation of the muscle causes a large-scale diffusion of Ca^{2+} from the sarcoplasmic reticulum to the sarcoplasm. Ca^{2+} then binds to the protein troponin C of myofibrils and produces an allosteric conformational change in the actin-tropomyosin-troponin complex; consequently, troponin I no longer assists tropomyosin to block the actin-myosin interaction. This initiates the actin-myosin reaction and the muscle fibre contracts. During relaxation, sarcoplasmic Ca^{2+} is again lowered by the active transport and sequestration of Ca^{2+} into the sarcoplasmic reticulum. This removes Ca^{2+} from troponin C and again enables tropomyosin to inhibit the actin-myosin interaction.

(b) In contracting *smooth muscles*, the rise in sarcoplasmic Ca^{2+} leads to its binding with the protein *calmodulin*. Ca^{2+} -calmodulin then binds to myosin light-chain kinase to activate it; the latter phosphorylates the p-light chain of myosin and blocks its inhibitory effect on actin-myosin interaction. This initiates that interaction for muscle contraction. During relaxation, the fall in sarcoplasmic Ca^{2+} leads to a dissociation of Ca^{2+} from calmodulin. The latter consequently fails to activate the light-chain kinase; this

restores the inhibitory action of p-light chain on actin-myosin interaction, leading to relaxation.

6. *Permeability of gap junctions*: Gap junctions form communicating channels between adjacent cells of cardiac muscle, visceral smooth muscle, bone, lens and nerve tissue. These channels allow intercellular flow of inorganic ions and small polar molecules. Gap junctions get closed when intracellular Ca^{2+} concentration exceeds 5×10^{-5} M. On the contrary, fall in intracellular Ca^{2+} below 10^{-7} M opens the gap junctions. By regulating the permeability of gap junctions in this way, Ca^{2+} controls the cell-to-cell transmission of nutrients and informations.

7. *Membrane permeability*: Ca^{2+} reduces membrane permeability to ions and water, probably by binding with calmodulin of cell membranes and consequently changing the conformation and hydration of membrane proteins.

8. *Neuromuscular excitability*: Ca^{2+} reduces neuromuscular excitability by reducing membrane permeability. A fall in serum Ca^{2+} raises the neuromuscular excitability producing spasms of muscles in hands, feet, face and larynx (*hypocalcemic tetany*).

9. *Special role in cardiac contraction*: Cardiac muscle differs from striated muscle in some roles of Ca^{2+} . T tubules of cardiac muscle fibres store large amounts of Ca^{2+} in combination with polyanionic mucopolysaccharides. For a period from the onset of each spike potential till 0.2-0.3 sec after the spike, considerable amounts of Ca^{2+} are released into sarcoplasm from T tubules in addition to the Ca^{2+} released from sarcoplasmic reticulum. This prolonged Ca^{2+} influx into the sarcoplasm causes a prolonged decrease in K^+ permeability of the membrane and a consequent continuation of membrane depolarization, resulting in a plateau after the

initial spike potential. At the end of the plateau, Ca^{2+} is again actively pumped into the sarcoplasmic reticulum and T tubules, lowering the sarcoplasmic Ca^{2+} concentration to raise the membrane permeability to K^+ and to cause membrane repolarization.

Rise in the extracellular fluid Ca^{2+} increases myocardial contractility and the force of cardiac contraction, but reduces the diastolic relaxation—this action is counterbalanced by the opposite action of K^+ . A fall in serum Ca^{2+} delays ventricular repolarization and slows the heart. Ca^{2+} -rich perfusion fluids may stop the heart in a contracted condition (*Calcium rigor*). These may be explained by the fact that a rise in Ca^{2+} concentration of ECF causes a proportionate rise in Ca^{2+} concentration in T tubules as these tubules are filled with ECF.

10. *Role in hormone action*: Ca^{2+} acts as the *second messenger* to mediate in the cellular actions of many hormones. Such hormones enhance the cytosol Ca^{2+} in the target cell—they either increase the transmembrane Ca^{2+} -influx from ECF or enhance the intracellular cAMP which in turn increases the translocation of Ca^{2+} from mitochondria to cytosol. Ca^{2+} then binds to the protein *calmodulin* in cellular membranes and cytosol to form Ca^{2+} -calmodulin complexes which modulate enzymatic, endocytotic and membrane activities of the target cell. Ca^{2+} also stimulates the secretion of protein hormones like insulin, stored in granules of endocrine cells, by stimulating microfilament contraction and thereby enhancing emiocytosis.

11. *Role in milk secretion*: About 250-300 mg of calcium are secreted in the daily milk output. Bone calcium may be depleted in Ca^{2+} -deficient lactating mothers, producing osteomalacia.

Functions of phosphorus:

1. *Constituent of bones and teeth*: Vide *Functions of calcium*.

2. *Energy transfer*: The free energy produced by metabolic reactions may be stored as high-energy phosphates like adenosine triphosphate and creatine phosphate, and may be liberated by their subsequent hydrolysis for utilization in other energy-requiring processes. High-energy phosphates are also utilized for phosphate transfers.

3. *Acid-base regulation*: Mixtures of dibasic and monobasic phosphates (HPO_4^{2-} and H_2PO_4^-) constitute the phosphate buffer which comes next to bicarbonate buffer in importance in maintaining the pH in extracellular fluids, and is also of considerable importance inside the cells except erythrocytes.

4. *Phosphorylation and phosphorolysis*: Phosphorylation is essential in reactions like glycolysis, glycogenesis, synthesis of triglycerides and nucleotides, and reversible covalent modifications of many enzymes. Phosphorolysis is the cleavage of a covalent bond in a substrate with the help of phosphoric acid. This happens in many metabolic pathways; e.g., phosphorolysis of glycogen to glucose 1-phosphate molecules in glycogenolysis, and that of purine ribonucleosides into free purines and ribose 1-phosphate in purine catabolism.

5. *Enzyme action*: Phosphate is present in nucleotides, many of which function as coenzymes; e.g., thiamin pyrophosphate, pyridoxal phosphate, nicotinamide coenzymes, flavin coenzymes and coenzyme A. Besides, reversible phosphorylation and dephosphorylation modify the actions of many enzymes such as glycogen phosphorylase, glycogen synthase, pyruvate dehydrogenase and adipose tissue lipase.

6. *Constituent of phosphoproteins, nucleic acids, phospholipids and lipoproteins*.

Absorption: Calcium is actively absorbed mainly from the duodenum and the first half of jejunum against electrical and concentration gradients and with the help of a

calcium-binding protein and a Ca^{2+} -dependent ATPase. Phosphates are absorbed as inorganic phosphates from the middle third of jejunum. Bile salts may help by including phosphates of calcium in water-soluble molecular aggregates (*micelles*) in the intestine. Other influencing factors are as follows:

1. *Ca : P ratio*: If the amount of either calcium or phosphate in the food exceeds double the amount of the other, intestinal absorptions of both are reduced due to the formation of insoluble complexes— $\text{Ca}(\text{H}_2\text{PO}_4)_2$, CaHPO_4 and $\text{Ca}_3(\text{PO}_4)_2$ of food have solubilities in the decreasing order. Absorption is maximal when food contains almost equal amounts of readily available calcium and phosphorus.

2. *Sugars*: Organic acids, produced by microbial fermentation of sugars in the intestine, increase the solubility of calcium salts and enhance their absorption.

3. *Proteins*: A high-protein diet increases calcium absorption because amino acids increase the solubility of calcium salts. Lysine and arginine from basic proteins cause maximal absorption of calcium.

4. *Fatty acids*: Failure of intestinal absorption of fatty acids reduces absorptions of both calcium and phosphate, because fatty acids form insoluble calcium soaps and thereby depress the ratio of available calcium and phosphorus in the intestine.

5. *Oxalates, phytates and iron*: Oxalates of spinach and colocasia form insoluble calcium oxalate. Food iron may form insoluble ferric phosphates. These respectively lower and raise the Ca : P ratio in the ileum beyond the range for optimal absorption. Phytic acid of cereals forms insoluble calcium phytates, reducing absorptions of calcium and phosphate.

6. *Vitamin D and parathormone*: A fall in serum Ca^{2+} stimulates parathormone secretion; parathormone then promotes the

conversion of vitamin D₃ to 1,25-dihydroxy-cholecalciferol (1,25-DHCC). But a fall in serum phosphate directly promotes the conversion of D₃ to 1,25-DHCC. The latter increases the synthesis of a Ca²⁺-carrier protein in the intestinal mucosa cells; this protein enhances Ca²⁺ absorption by transporting Ca²⁺ across the intestinal cell membrane. Vitamin D increases phosphate absorption also.

Calcium balance : During pregnancy and growth, the absorbed calcium is largely retained in developing bones so that calcium intake exceeds its outflow in urine and feces (*positive calcium balance*). When the amount of absorbed calcium exactly equals the amount excreted, the body is in *calcium equilibrium*. Hypersecretion of parathormone, deficiency or hypervitaminosis of vitamin D, prolonged zero-gravity during space flights, old-age osteoporosis or prolonged bed confinement induces *negative calcium balance* by increasing calcium excretion above the calcium intake.

Distributions : Adult human body contains about 1200 and 600 g of calcium and phosphorus, respectively. About 99% of the calcium and 80% of the phosphorus remain in bones and teeth. In the skeletal system and extracellular fluids, calcium predominates over magnesium; reverse is the situation in erythrocytes, skeletal muscles, spleen and epithelia (Table 6.1). Plasma carries almost all the calcium of blood. Each dl (100 ml) of plasma contains 9-11 mg of calcium in total; of this, *ionized Ca²⁺* and *non-diffusible protein-bound calcium* (mainly Ca-albumin complexes) amount to 4.5-5.5 mg each while *diffusible non-ionic calcium* amounts to 0.4-0.6 mg only. Each dl of whole blood carries about 40-50 mg of total phosphorus, more in the blood cells than in the plasma. Each dl of plasma contains in adults about 3-5 mg of *inorganic phosphates* and about 9-10 mg of *lipid phosphorus*, mainly existing as phospholipids. *Acid-soluble phosphorus*

TABLE 6.1. *Distributions of Ca²⁺, Mg²⁺ and PO₄³⁻.*

<i>Tissue/fluid</i>	<i>Concentrations in mg per 100 ml or 100 g</i>		
	Calcium	Magnesium	Phosphorus
Skeletal muscle	7	19-21	160
Cardiac muscle	6	13	145
Spleen	10	12	170
Nerve	15	10	300
Bone	11000	180	5000
Dentin	27000	790	12500
Dental enamel	36000	470	17500
Blood	5-6	2-4	40-50
Plasma	9-11	2-3	12-18
CSF	4.5-5.5	2.5-3.5	1.4-3

includes inorganic phosphates and non-lipid, non-nucleoprotein organic phosphoesters like ATP and hexose phosphates; it amounts to about 25-30 mg per dl of whole blood.

Transport across mitochondrial membranes : Ca²⁺-carrier proteins of the inner mitochondrial membrane actively transport Ca²⁺ from the cytosol to the mitochondrial matrix against a concentration gradient, utilizing the membrane potential but requiring no simultaneous exchange with any cation. Such active mitochondrial collection of Ca²⁺ happens in the liver, bones and diastolic cardiac muscle. It maintains the cytosol Ca²⁺ at 1 micromolar concentration or lower and assists in the diastolic fall of sarcoplasmic Ca²⁺ in the cardiac tissue. Ca²⁺ outflow across the inner membrane is slow and passive, and occurs down a concentration gradient. A phosphate-hydroxide carrier of the inner mitochondrial membrane also transports H₂PO₄⁻ ions into the mitochondria against a concentration gradient and in exchange of OH⁻ ions.

Excretion : About 200-300 mg Ca may be excreted in the daily urine. 98% of the calcium of glomerular filtrate is actively reabsorbed mainly in the proximal tubule and

to a smaller extent, in the distal and collecting tubules. About 70% of the phosphates of glomerular filtrate is actively reabsorbed in tubules; phosphorus is mainly excreted in urine ($0.8-1.4 \text{ g day}^{-1}$) as inorganic dibasic and monobasic phosphates. Small amounts of phosphates and as much as $0.8-1 \text{ g}$ of calcium are excreted in the feces every day. Fecal calcium comes from both food and gastrointestinal secretions.

Regulation :

1. *Vitamin D* : (a) 1,25-Dihydroxycholecalciferol (1,25-DHCC) and to a lesser extent, 24,25-DHCC increases *intestinal absorptions* of calcium and phosphates, and (b) promotes hydroxyapatite synthesis and *mineral deposition in bones*. (c) 1,25-DHCC also increases *phosphate reabsorption* in the renal tubules.

2. *Ca : P ratio in blood* : The rise of one of these ions in blood is usually accompanied by a fall of the other.

3. *Plasma proteins* : A fall in plasma proteins may lower the protein-bound calcium and the total blood calcium, without altering the concentrations of ionized Ca^{2+} and diffusible non-ionic calcium.

4. *Calcitonin* : This hormone lowers serum calcium and phosphate by reducing their mobilization from bones, and maintains the bone minerals particularly in growing animals.

5. *Parathormone* : It increases serum Ca^{2+} and lowers serum phosphates in several ways :

(a) Parathormone (PTH) increases *intestinal absorptions* of calcium and phosphorus by promoting the formation of 1,25-DHCC from vitamin D_3 .

(b) PTH reduces the *reabsorption of phosphates* by the proximal tubule of kidney and thus increases urinary phosphates.

(c) PTH may retain calcium in the body by promoting *reabsorption of calcium* in the ascending thick limb of Henle's loop.

(d) PTH increases *resorption of bones* by both osteoclasts and osteocytes, and enhances mobilization of Ca^{2+} and PO_4^{3-} from bones.

6. *Sex hormones* : Estrogens and testosterone promote *retention and deposition* of calcium in bones. In women, deficiency of bone matrix, osteoporosis, delayed recovery from fractures and negative calcium balance are sometimes observed after menopause; estrogens and testosterone bring relief.

Clinical conditions : *Rickets and osteomalacia* are produced by the dietary deficiency of calcium, phosphorus or vitamin D and are characterized by reduced calcium and phosphate absorption, low serum Pi, rise in urinary phosphates, demineralization of bones and negative calcium balance; serum calcium is, however, almost normal in rickets although sometimes it falls in osteomalacia. *Hyper-vitaminosis D* may raise the serum calcium. *Hyperparathyroidism* causes demineralization of bones, rise of serum calcium, fall in serum Pi, high urinary calcium and phosphate, and metastatic calcifications of soft tissues. *Hypoparathyroidism* reduces the serum calcium, urinary calcium and urinary Pi, raises the serum Pi moderately and produces hypocalcemic tetany.

Vitamin D-resistant rickets and idiopathic osteomalacia are due to an X-linked genetic disorder of phosphate metabolism leading to reduced intestinal and renal absorptions of phosphates, low serum phosphate, high serum alkaline phosphatase activity, high urinary Pi and poor mineralization of bones.

6.3 MAGNESIUM METABOLISM

Sources : Cereals, nuts, dry peas, beans, soybeans, chocolate.

Daily requirements : Adult man : 350 mg ; adult woman : 300 mg ; pregnant or lactating woman : 450 mg ; infants : 50-70 mg ; children : 150-250 mg.

Functions :

1. *Constituent of bones and teeth* : About 16 g of magnesium or 70% of the body magnesium is present as apatites in bones, dental enamel and dentin.

2. *Neuromuscular excitability* : Mg^{2+} reduces neuromuscular excitability and counteracts the excitatory effects of monovalent cations. Low serum magnesium and increased neuromuscular excitability (*hypomagnesemic tetany*) occur in rats and dogs on a low-magnesium diet, in cattle grazing in low-magnesium areas, and in men suffering from acute diarrhoea or chronic alcoholism. Excessive administration of magnesium salts may increase serum magnesium and depress neuromuscular excitability, resulting in anaesthesia.

3. *Enzyme action* : Mg^{2+} is a cofactor for numerous enzymes like AmLev synthetase, transketolase, phosphoglucomutase, squalene synthetase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. It is an essential cofactor for enzymes using ATP, e.g., glucokinase, carbamoyl phosphate synthetase, phosphofructokinase, mevalonate kinase, fatty acid thiokinase, argininosuccinate synthetase, pyruvate carboxylase, ATP sulfurylase, methyladenosyl transferase and adenosyl-5'-phosphosulfate 3'-phosphokinase ; Mg^{2+} first binds with the β and γ phosphates of ATP to form Mg^{2+} -ATP chelate which then binds with the enzyme. Either Mg^{2+} -ADP or Mg^{2+} -PPi is liberated as one of the products.

Absorption and fate : Magnesium is absorbed from small intestine. Vitamin D, food oxalates and phytates, unabsorbed fatty acids, sugars and proteins of food, and acidic intestinal pH affect the absorption of magnesium, similar to calcium absorption. Urinary magnesium averages 7-25 mg dl⁻¹.

Magnesium is present in higher concentrations in cells than in the ECF (Table 6.1). About one-fifth of plasma magnesium is trans-

ported in combination with plasma proteins while 1.5-2.5 mg dl⁻¹ may exist as free Mg^{2+} .

Clinical conditions : Hypermagnesemia may be produced due to magnesium retention in renal failures. Primary aldosteronism lowers serum Mg^{2+} as urinary Mg^{2+} rises due to excessive amounts of aldosterone secreted. Diarrhoea, chronic alcoholism, dietary magnesium deficiency or acute pancreatitis may also reduce serum Mg^{2+} , sometimes producing hypomagnesemic tetany, tremor and semi-coma.

6.4 SULFUR METABOLISM

Sources and requirements : Meat, fish, egg, cartilage, liver, brain, spinach and lettuce supply sulfur. Adequate intake of proteins fulfils sulfur requirements also, because sulfur is present mainly in S-containing amino acids.

Functions :

1. *Detoxication* : Compounds possessing phenolic hydroxyl groups (e.g., phenol, skatole, indole and steroids) may be detoxicated in the liver by conjugation with SO_4^{2-} ions from S-containing amino acids. Aromatic hydrocarbons and their derivatives (e.g., bromobenzene) are detoxicated by their conjugation with acetylated cysteine.

2. *Sulphydryl enzymes* like triose phosphate dehydrogenase, papain and urease depend on free SH groups at their active sites for activities.

3. *Some non-heme iron enzymes* like iron-sulfur proteins of the mitochondrial electron transport chain contain iron and sulfur, but no free SH in the prosthetic group.

4. *S-Adenosylmethionine* is the coenzyme for methylferases. It donates the CH_3 group carried on its sulfur to substrates like nicotinamide to methylate them (Fig. 6.2).

5. *SH group of glutathione* donates H^+ and electron for many reductions.

6. *SH groups of coenzyme A and acyl carrier protein (ACP)* form fatty acid

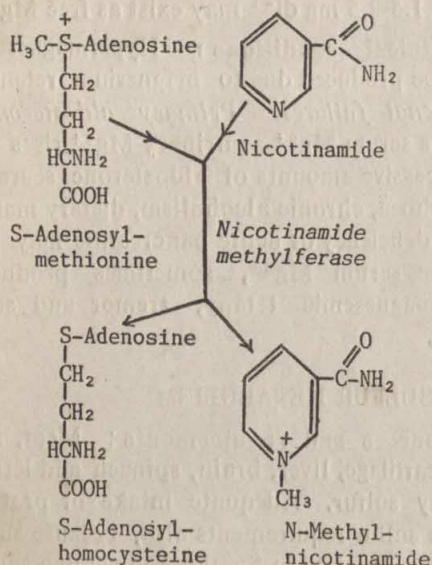


Fig. 6.2. Action of S-adenosylmethionine.

thioesters (e.g., acetyl-CoA and acyl-ACP) for the metabolism and transfer of acyl groups.

7. Adenosine-3'-phosphate-5'-phosphosulfate ('active' sulfate) is formed in the liver from ATP and sulfate by ATP sulfurylase and adenosine-5'-phosphosulfate 3'-phosphokinase (Fig. 6.3). Its sulfate group is transferred to other substrates like hexosamines. S-Adenosylmethionine, active sulfate and acyl complexes of coenzyme A and ACP are *high-energy sulfur compounds*.

8. *Sulfated hexosamines* and *hexuronic acids* are important constituents of mucopolysaccharides and contribute negative charges and acidic properties to the latter. Sulfated galactose occurs in sulfolipids and some mucopolysaccharides.

9. *S-containing vitamins* (biotin and thiamin) form some coenzymes in the body.

10. *Protein structure*: Cysteine-SH of proteins may form intrachain and interchain S-S linkages and may thereby contribute to the tertiary and quaternary structures of proteins.

Distribution: Of about 150 g of body sulfur, most remain in proteins as S-containing amino acids. Small amounts of inorganic sulfates occur in tissues and body fluids. 100 ml of blood contain 0.1-1 mg sulfur as *inorganic sulfate* and about 2.3-5.5 mg sulfur in organic compounds; organic sulfur consists of *neutral sulfur* (2.4-4 mg dl⁻¹) which mainly comprises free S-containing amino acids, and *etheral sulfates* (0.1-1.1 mg dl⁻¹) which include detoxication products like indoxyl and skatoxyl sulfates. Glutathione and ergothioneine are organic sulfur compounds present in RBC and liver.

Fate: S-containing amino acids produce inorganic sulfates during catabolism. Liver conjugates inorganic sulfates with aromatic compounds to produce etheral sulfates. Sulfur is excreted in urine as inorganic sulfates (0.7-1 g day⁻¹), neutral sulfur (0.07-0.12 g) and etheral sulfates (0.07-0.16 g).

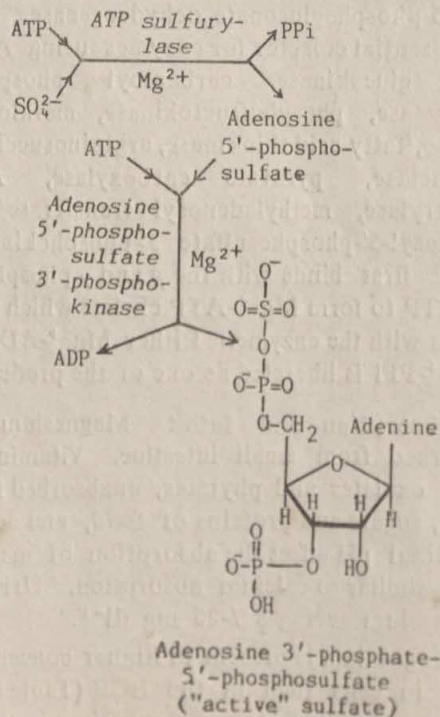


Fig. 6.3. Biosynthesis of 'active' sulfate.

6.5 SODIUM, POTASSIUM AND CHLORINE METABOLISMS

Sources : Tablesalt, pickles, butter, cheese, salted fish or meat, salted nuts and khoa contain sufficient NaCl. Unrefined salt, sugarcane molasses, pineapple, orange, banana, date, potato, cabbage, chicken, pork and dried apricots are rich in potassium.

Daily requirements : *Sodium* : adults : 1-3.5 g ; infants : 0.1-0.5 g ; children : 0.3-2.5 g. *Chloride* : adults : 1.5-5 g ; infants : 0.25-1 g ; children : 0.5-4 g. *Potassium* : adults : 1.8-5.5 g ; infants : 0.35-1.2 g ; children : 0.5-4.5 g.

Distributions : Adult human body contains about 110-130 g potassium, 80-90 g sodium and 70-80 g chlorine. Na^+ and Cl^- concentrations are much greater in the extracellular fluid (ECF) than inside the cell ; the case is the reverse for K^+ . Na^+ and K^+ are the principal cations in the ECF and cells respectively ; Cl^- is the chief anion in the ECF and a major mineral anion in cells.

Cl^- tends to move inward across the cell membrane due to higher Cl^- concentration outside the membrane. This is counter-balanced by the outward movement of Cl^- due to the relative negativity of the cell interior.

TABLE 6.2. *Distributions of Na^+ , K^+ and Cl^- .*

Tissue/fluid	Concentration in mg per 100 g or 100 ml		
	Sodium	Potassium	Chloride
Whole blood	160-170	180-210	250-300
Plasma	320-340	17-22	330-350
CSF	340-355	14-16	420-440
Lymph	315-360	15-20	330-430
Muscle	80-120	250-350	45-50
Nerve tissue	300-310	500-535	160-175
Sweat	30-300	20-120	50-300
Saliva	70-230	35-80	140-250
Tear	320-340	14-20	120-135

$\text{Na}^+\text{-K}^+$ pump : Intracellular and extracellular Na^+ concentrations average 15 and 150 mM respectively ; this high inward concentration gradient of Na^+ is opposite to what is expected from the Gibbs-Donnan effect of much higher concentrations of nondiffusible protein and phosphate anions inside the cell than outside it. Intracellular and extracellular K^+ concentrations average 100 and 5 mM respectively ; such high magnitude of the outward K^+ gradient cannot be explained by either the Gibbs-Donnan effect of intracellular nondiffusible anions or the far higher membrane permeability to K^+ than to Na^+ . A membrane mechanism called the $\text{Na}^+\text{-K}^+$ pump (sodium pump) maintains both magnitudes and directions of transmembrane concentration gradients of those ions. This pump consists of a tetrameric membrane glycoprotein, $\text{Na}^+\text{-K}^+\text{-ATPase}$ (MW 270000). Its activity depends on the presence of Na^+ and K^+ on respectively the cytoplasmic and extracellular sides of the membrane, and requires Mg^{2+} as a cofactor. The enzyme hydrolyzes a high-energy phosphate bond of ATP and uses that energy to transport three Na^+ ions outward and simultaneously two K^+ ions inward across the membrane. In this way, each $\text{Na}^+\text{-K}^+$ pump transfers every minute about 9000 Na^+ ions outward and about 6000 K^+ ions inward across the plasma membrane. $\text{Na}^+\text{-K}^+\text{-ATPase}$ is highly active in cells whose activities depend largely on transmembrane Na^+ fluxes ; e.g., neurons, muscle fibres, renal tubule cells, intestinal mucosa cells, salivary and sweat gland cells, and cells of electric organs of eels and nasal or orbital salt glands of marine birds and reptiles. Erythrocytes show relatively less activity of this enzyme. On average, about 250 $\text{Na}^+\text{-K}^+$ pumps occur on the membrane of an erythrocyte.

$\text{Na}^+\text{-K}^+\text{-ATPase}$ probably exists in two forms, E_1 and E_2 . The E_1 form presents its ion-binding and phosphate-binding sites on the cytoplasmic surface of the membrane. Three

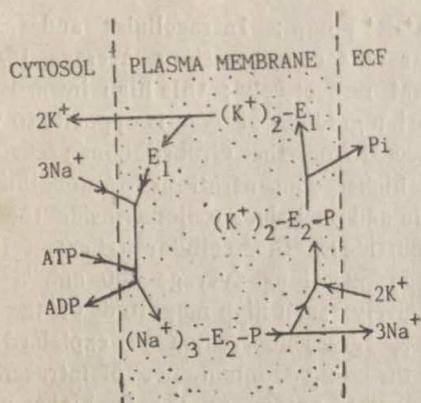


Fig. 6.4. Transmembrane Na⁺ transport by Na⁺-K⁺-ATPase (E₁ and E₂ forms).

Na⁺ ions from the cytoplasm bind with the ion-binding sites of the E₁ form of ATPase. This leads to the phosphorylation of the sidechain of a specific aspartate residue of the enzyme with the help of cytoplasmic ATP and in presence of Mg²⁺ (Fig. 6.4). The conformation of the E₁ form consequently changes to give the E₂ form of the enzyme. This brings both ion-binding and phosphate-binding sites on the extracellular surface of the membrane, lowers the affinity of the ATPase for Na⁺ and releases the latter in the extracellular fluid (ECF). On the contrary, two K⁺ ions from ECF get bound now to the ion-binding sites of the enzyme. This binding with K⁺ lowers the affinity of the ATPase for phosphate and releases the latter in the ECF. This dephosphorylation changes the conformation of ATPase to its E₁ form again, bringing its ion-binding sites to the cytoplasmic surface of the membrane and lowering the affinity for K⁺. It results in the release of the K⁺ ions from the ATPase into the cytoplasm.

Cardiotonic steroids like ouabain and digitalis, when present outside the cell, inhibit the Na⁺-K⁺ pump by blocking the dephosphorylation step. Vanadate inhibits the pump, when present inside the cell.

Absorption of sodium : Intestinal absorption of food Na⁺ and renal reabsorption of

urinary Na⁺, both depend on the Na⁺ pump of basal and lateral plasma membrane of intestinal and renal cells. Na⁺ pump actively extrudes Na⁺ ions into the extracellular fluid across the basal and lateral plasma membrane of those cells (Fig. 6.5). This keeps the Na⁺ concentration lower in the cell than in the intestinal or renal tubular lumen. Na⁺ consequently diffuses into the cell across its luminal or microvillus membrane from the lumen. Ouabain, cold or absence of O₂ depresses the active transport of Na⁺ from the mucosal (luminal) side to the serosal side of an excised ileum preparation by inhibiting the sodium pump.

This active absorption of Na⁺ is frequently coupled with the absorption of glucose or amino acids (*symport*). The low intracellular Na⁺ concentration, caused by the sodium pump of the basal and lateral plasma membrane of intestinal cell, leads to the diffusion of Na⁺ from the intestinal lumen across the luminal plasma membrane into the cell (Fig. 6.6). But while crossing the luminal plasma membrane, Na⁺ binds on its extracellular surface with a membrane protein acting as a carrier. This changes the conformation of the latter to expose its glucose-binding sites on

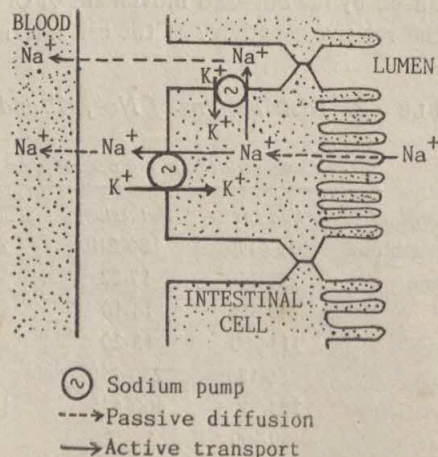


Fig. 6.5. Active absorption of Na⁺ from intestinal lumen.

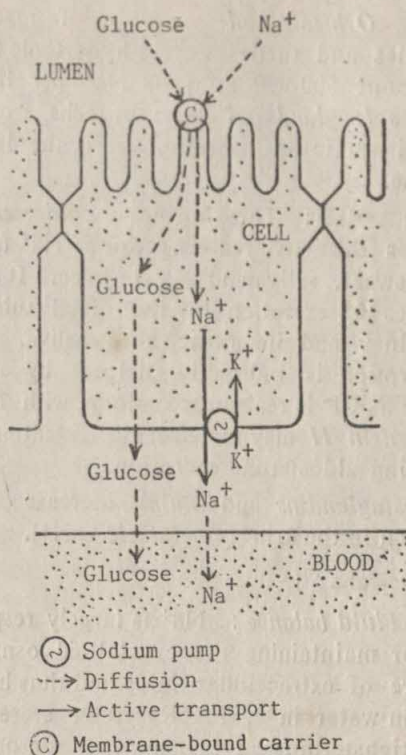


Fig. 6.6. Symport of glucose and Na^+ by intestinal cell.

the membrane surface. The Na^+ -bound carrier consequently binds with glucose from the lumen to form a ternary Na^+ -carrier-glucose complex in the membrane. As the carrier-bound Na^+ diffuses through the membrane to reach its cytoplasmic surface, Na^+ dissociates from the carrier to enter the cytoplasm. This changes the conformation and reduces the glucose affinity of the carrier (Fig. 6.7).

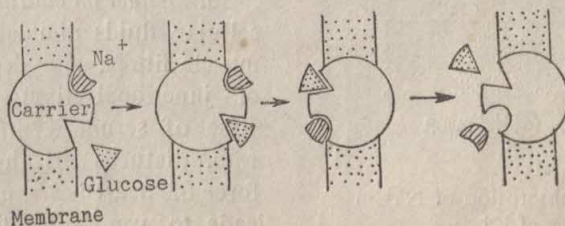


Fig. 6.7. Model of Na^+ -glucose symport.

Consequently, glucose also dissociates from the carrier to enter the cytoplasm in spite of a higher glucose concentration there. In this way, the energy released by the down-gradient flow of Na^+ is utilized in the active absorption of glucose or amino acids.

In the renal tubules, the active reabsorption of Na^+ by the Na^+ - K^+ -ATPase is coupled with the elimination of K^+ in the tubular lumen (Fig. 6.8). In the tubules, Na^+ may be actively reabsorbed in exchange of the elimination of H^+ also in the tubular urine.

Excretion: About 3-5 g sodium, 1.5-3 g potassium and 7-8 g chloride are excreted in 24 hours' urine. About 70% of the Na^+ , filtered into the Bowman's capsule, is reabsorbed from the proximal convoluted tubule and another 27% from the distal convoluted tubule and the ascending limb of Henle's loop. In the convoluted tubules, Na^+ is actively reabsorbed against electrochemical gradients. But in the ascending limb of Henle's loop, Na^+ is reabsorbed by diffusion. Cl^- is reabsorbed by diffusion from the proximal and distal tubules and the thin segment of the ascending limb along the electrical gradient developed by the reabsorption of Na^+ . Some Cl^- is also actively reabsorbed from the thick segment of the ascending limb. Almost all the filtered K^+ is actively reabsorbed in the proximal tubule and the ascending limb. The urinary K^+ is mostly secreted into the distal tubule in exchange of the reabsorbed Na^+ —this *obligatory loss* of K^+ in the urine amounts to about 160 mg day^{-1} .

Some Na^+ , K^+ and Cl^- are secreted in saliva, gastric juice, pancreatic juice, tear, sweat, etc. During profuse sweating, the largest amounts of Na^+ and Cl^- losses are accounted for by the sweat. On thermal acclimatization, secretion of aldosterone increases significantly and this hormone reduces the Na^+ and Cl^- concentrations in sweat. The *obligatory loss* of Na^+ from the human body is less than 200 mg day^{-1} .

Specialized salt glands: Marine birds and reptiles drink hyperosmotic sea-water. Marine elasmobranchs acquire much NaCl from sea-water through their integuments and from the marine invertebrates they feed upon. Non-mammals except birds cannot secrete hypertonic urine and so, cannot eliminate this excess salt through urine; even avian urine is not hypertonic enough to get rid of the salts gained by drinking sea-water. Marine birds and reptiles are further handicapped by the small volumes of their urine. *Nasal salt glands* of marine birds like penguins, pelicans and gulls secrete per hour about 10-80 ml of concentrated NaCl solutions (about 500-850 mEq of Na^+ per litre) into the nasal canals to eliminate the excess NaCl . Similar oral

salt glands open into the mouth of marine snakes. *Orbital salt glands* of marine crocodiles and turtles secrete hypertonic tear with about 500-900 mEq of Na^+ per litre. *Rectal salt glands* of elasmobranchs secrete almost isotonic NaCl solutions into the large intestine.

Regulation: The hormone *aldosterone* increases the active reabsorption of Na^+ from urine, sweat, saliva and gastric juice. It also increases K^+ excretion by the distal tubules of kidney and in sweat and saliva. Cl^- reabsorption is indirectly helped by aldosterone as Cl^- is reabsorbed along with Na^+ . *Angiotensin II* may increase Na^+ retention by enhancing aldosterone secretion.

Prostaglandins and *kinins* increase Na^+ excretion in the urine (*natriuretic effect*).

Functions :

1. **Fluid balance :** Na^+ is largely responsible for maintaining the crystalloid osmotic pressure of extracellular fluids and thus helps to retain water in ECF. Excessive secretion of corticosteroids in primary aldosteronism and Cushing's syndrome causes Na^+ retention, high plasma Na^+ and increased ECF volume; in Addison's disease, failure of corticosteroid secretion increases the loss of Na^+ and lowers plasma Na^+ and ECF volume. Potassium maintains the intracellular crystalloid osmotic pressure and retains fluid within cells.

2. **Neuromuscular excitability :** The monovalent cations, Na^+ and K^+ , increase the neuromuscular excitability; it is counter-balanced by the opposite effect of Ca^{2+} and Mg^{2+} .

3. **Effect on cardiac muscle :** K^+ of extracellular fluids stimulates relaxation of cardiac muscle fibres, and depresses the excitability of junctional tissues, atria and ventricles. Rise of serum K^+ (*hyperkalemia*) slows the rate, disturbs the rhythm and weakens the force of heart-beats, prolongs the diastole and leads to ventricular fibrillation and cardiac

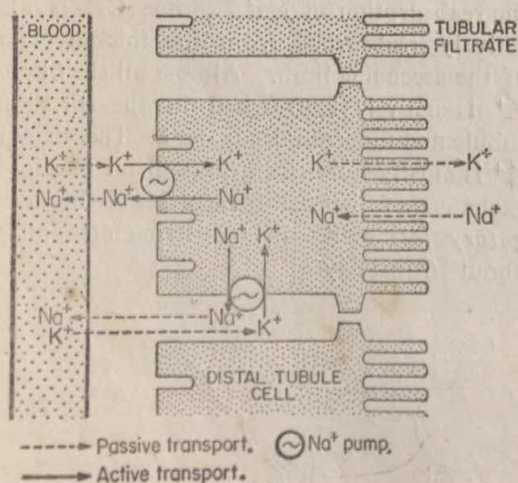


Fig. 6.8. Renal reabsorption of Na^+ in exchange of K^+ .

arrest in diastole. The P wave of ECG is weakened, indicating a depression of atrial depolarization; the QRS duration is prolonged, indicating slower ventricular depolarization; the T wave of repolarization is elevated. Rise in K^+ concentration in the extracellular fluid reduces the negativity of the resting membrane potential, leading to a fall in the strength of the action potential and consequently in the force of the cardiac contraction. On the other hand, low serum K^+ (*hypokalemia*) lengthens the PR interval indicating slower atrial conduction, reduces the ECG voltages, depresses ST components, flattens or inverts the T and ultimately causes cardiac arrest in systole. Excess of Na^+ in the extracellular fluid can also depress cardiac contractility by competing with Ca^{2+} and reducing the effectiveness of the latter in the activation-contraction mechanism of the cardiac muscle fibre. The transmembrane balance between the extracellular Na^+ and the intracellular K^+ maintains the normal rhythmicity and excitability of cardiac muscle.

4. *Acid-base balance*: Na^+-H^+ exchange in the renal tubules helps to acidify the urine. Cl^- has a role in buffering CO_2 ; some of the HCO_3^- , formed from CO_2 in RBC, passes out into the plasma in exchange of Cl^- which enters the erythrocytes (*chloride-shift phenomenon*). If intracellular and serum K^+ concentrations are depressed by low K^+ intake, cells are obliged to retain H^+ in place of K^+ and intracellular acidosis results; simultaneously, Na^+-K^+ exchange is largely replaced by Na^+-H^+ exchange in renal tubules, lowering the urinary pH and producing alkalosis of extracellular fluids.

5. *Cl^- in HCl secretion*: Plasma Cl^- is the source of Cl^- for gastric HCl.

6. *Role in resting membrane potential*: Plasma membrane has a poor Na^+ permeability and the passive Na^+ inflow through it is far lower than the active Na^+ outflow from the cell by the membrane-bound sodium pump. So,

Na^+ concentration is ordinarily maintained far higher outside the cell membrane than inside it. Nondiffusible protein anions and organic phosphate anions in the cell and the excess of Na^+ outside create considerable intracellular electronegativity. This causes passive influx of K^+ . The sodium pump (Na^+-K^+ pump) also pumps in some K^+ in exchange of Na^+ pumped out. Consequently, K^+ concentration is higher inside than outside the plasma membrane. But for each pair of K^+ ions actively transported into the cell by the Na^+-K^+ pump, three Na^+ ions are pumped out. This leads to a slight excess of cations outside the membrane while a slight excess of anions is created inside the cell due to the nondiffusible anions. This separation of charges by the membrane is called the *polarization* of the membrane. It creates a potential difference of -70 to -95 millivolts across the membrane (*resting membrane potential*).

7. *Role in action potential*: The membrane of the nerve or muscle fibre is locally depolarized by stimulation. This rapidly increases manifold its permeability to Na^+ , causing considerable transmembrane influx of Na^+ down its inward concentration gradient and towards the relatively electronegative cell interior. K^+ permeability rises slower and later; so, initially the transmembrane K^+ outflow down its own outward concentration gradient falls far short of the Na^+ influx. This unbalanced excess of Na^+ influx progressively increases the depolarization of the membrane, reversing the electronegativity on its inner side to a positivity and raising the membrane potential from its resting value of -70 mV to a peak of $+35$ to $+45$ mV; this creates the *spike potential*. The Na^+ permeability soon declines, reducing the Na^+ inflow; K^+ permeability rises simultaneously and increases the K^+ outflow. These restore the negativity on the inner side of the membrane, terminating the spike potential. The ion distributions are

subsequently restored and the membrane is repolarized by the sodium pump which actively transports the excess Na^+ to the exterior and in exchange carries the excess K^+ inward.

When a spike potential arrives or arises on a depolarized part of the membrane, the resulting potential difference with the adjoining polarized part of the membrane causes a flow of some cations from the depolarized to the polarized membrane through the axoplasm and a reverse flow of some cations through the interstitial fluid. This depolarizes the adjoining inactive membrane and initiates the generation of spike potential there. These events are repeated in subsequent segments of the membrane, conducting the spike potential.

The cardiac muscle fibre differs from the striated muscle fibre in showing a prolonged state of depolarization in the form of a plateau after the spike. The plateau results from a reduced membrane permeability to K^+ from the onset of the action potential till 0.2-0.3 sec after the spike. This reduces K^+ outflow through the membrane of the cardiac muscle fibre during that period and consequently prolongs depolarization of the membrane, producing the plateau. At the end of this period, membrane permeability to K^+ is enhanced, action potential ends and repolarization follows.

Clinical conditions: High urinary elimination of K^+ may produce *hypokalemia* (low serum K^+) and low intracellular K^+ in primary aldosteronism and Cushing's syndrome owing to excessive corticosteroid secretion. *Hypokalemia* also occurs due to severe vomiting, diarrhoea, malnutrition and low potassium diet. Rise in the heart rate, AV block, renal damage, polyuria, muscular weakness, paralysis, reduced glucose tolerance and a tendency to tetany may result. *Hyperkalemia* (high serum K^+) as well as low urinary K^+ occurs in Addison's disease due to poor corticosteroid secretion, and causes slowing

of the heart, muscular weakness, fall of blood pressure and depression of nervous system.

Intense vomiting may cause *Cl^- depletion* due to the loss of Cl^- with the gastric contents. Plasma Na^+ , Cl^- and K^+ levels fall, some of the lost Cl^- is replaced by HCO_3^- in the ECF and metabolic alkalosis may result. In Addison's disease, primary aldosteronism and Cushing's syndrome, serum Cl^- changes are similar to those of serum Na^+ .

Hypernatremia (rise in plasma Na^+) may be caused by Na^+ retention due to excessive secretion of either aldosterone (in primary aldosteronism) or glucocorticoids (in Cushing's syndrome); it leads to water retention, a rise in ECF volume and a consequent hypertension. Excessive elimination of dilute urine in diabetes insipidus or excessive loss of sweat (with NaCl concentration lower than that in plasma) may also lead to hypernatremia without any rise in the body sodium. *Hyponatremia* (low serum Na^+) arises from an increased loss of sodium in Addison's disease or after intense vomiting and diarrhoea; this leads to dehydration, fall in ECF volume, low blood pressure and loss of body weight. Hyponatremia with nausea, muscle cramps, headache and diarrhoea may also result from drinking salt-free fluids after excessive loss of sweat (*salt cramps*). Polyuria of nephritis and diabetes mellitus may cause excessive urinary loss of Na^+ , leading to hyponatremia.

6.6 TRACE ELEMENTS

These minerals are present in the body in much smaller amounts than sodium, potassium, chloride, calcium or phosphorus, and are required in the diet in amounts far lower than 100 mg day^{-1} . They include iron, copper, zinc, manganese, molybdenum, cobalt, selenium, iodine, fluorine and chromium.

6.7 IRON

Sources : Liver, lentils, lettuce, spinach, radish tops, egg-yolk, nuts, date, soyabean, molasses.

Daily requirements : Adult man : 25 mg ; adult woman : 30 mg ; pregnant woman : 40 mg ; lactating woman : 30 mg ; children : 20-25 mg.

Functions : Iron helps mainly in the transport, storage and utilization of oxygen in the body.

1. *Heme compounds* : Many iron-porphyrin-protein complexes or hemoproteins participate in the transport and storage of oxygen ; e.g., hemoglobin and myoglobin. Iron deficiency results in *hypochromic microcytic anemia* with low blood hemoglobin. *Heme enzymes* are hemoproteins which participate in electron transport and tissue oxidation ; e.g., cytochromes, catalases, peroxidases and hemoflavoproteins such as cytochrome b_2 .

2. *Non-heme iron enzymes (NHI)* : These electron carriers have iron in the prosthetic group, but no porphyrin. They include (a) *iron-sulfur-flavoproteins* such as succinate dehydrogenase and NADH dehydrogenase of mitochondria, and (b) *iron-sulfur-proteins* like adrenodoxin of adrenal cortex mitochondria and Fe-S-proteins of the mitochondrial electron transport chain.

Absorption : Normally about 1 mg of iron is absorbed from food every day. Soluble inorganic salts of iron are easily absorbed from the small intestine. Gastric acid liberates free Fe^{3+} from non-heme iron compounds. Vitamin C and glutathione of food help in iron absorption by reducing Fe^{3+} to less polymerizable and more soluble Fe^{2+} . Vitamin C and amino acids also help by forming soluble iron-ascorbate and iron-amino acid chelates. Oxalates, phosphates and phytates of food reduce iron absorption by forming insoluble complexes of iron. Heme of food is absorbed

as such in the intestine and is degraded in the intestinal mucosa cells to release its iron.

Fe^{2+} absorbed into the intestinal mucosa cell is oxidized to Fe^{3+} which combines with a water-soluble cytoplasmic carrier called *intracellular iron carrier*. The latter (i) delivers a fixed amount of iron to mitochondria, (ii) transfers some iron to another water-soluble cytosol protein called *apoferritin* (MW ≈ 500000) which binds the Fe^{3+} ions to form *ferritin*, and (iii) transfers some iron across the serosal cell membrane to a plasma β_1 -globulin called *apotransferrin* (MW ≈ 90000) which binds the Fe^{3+} ions to form *transferrin*. Ferritin is the *storage form of iron* in the intestinal mucosa cell and contains up to about 24% iron or 4300 Fe^{3+} ions per molecule as ferric hydroxide-ferric phosphate micelles. The intracellular iron carrier, on the other hand, holds Fe^{3+} in either protein-bound or chelated forms and represents the *carrier iron pool* in the intestinal cell. Presence of sufficient amounts of iron in the carrier iron pool keeps the intracellular iron carrier nearly or totally saturated and consequently reduces further iron absorption ; in iron deficiency, the intracellular iron carrier remains largely unsaturated and consequently increases iron absorption in the intestine.

Transport and fate : Fe^{3+} is enzymatically reduced to Fe^{2+} in the mucosa cell. Fe^{2+} then enters the plasma where it is reoxidized to Fe^{3+} by a copper-protein, called ceruloplasmin or *serum ferroxidase*. Fe^{3+} is then incorporated into a β_1 -pseudoglobulin, called *transferrin*. Transferrin is a glycoprotein, each molecule of which can bind two Fe^{3+} ions. Normally, 100 ml of blood contain about 300 mg transferrin. The latter may carry up to about 360 μg of iron, but ordinarily carries only 70-170 μg of iron (*protein-bound iron* or *BI*). The latter value is depressed in iron deficiency anemia.

Developing erythrocytes may draw iron from transferrin for hemoglobin synthesis.

Iron is stored in the liver, spleen and red bone marrow as two iron-protein complexes, ferritin and hemosiderin. *Hemosiderin* is a water-insoluble microscopic aggregate of denatured ferritin and contains about 37% iron as ferric hydroxide micelles; it is deposited in tissues particularly when ferritin is nearing its saturation with iron. Ferritin may hold 500-800 mg of iron in total, of which 9% is stored in the liver. During the uptake of Fe^{2+} from plasma, apoferritin itself acts as a ferroxidase to oxidize it to Fe^{3+} which then binds to apoferritin to form ferritin. When necessary, Fe^{3+} of ferritin is reduced to Fe^{2+} by a ferritin reductase of liver cells. Fe^{2+} is then released in the plasma.

Of the total body iron of 4 g, about 3 mg may be transported as transferrin in the plasma. Hemoglobin, myoglobin, and heme enzymes contain about 3 g, 140 mg and 10 mg of iron respectively. Only about 1-1.5 mg of iron is excreted daily by a human male. Most of that amount passes out in the feces; very little is excreted in the bile and only 50-70 μg appear in the urine. The major portion (>22 mg) of iron, released from catabolized hemoglobin, is recycled every day in the synthesis of heme and non-heme iron complexes while some is stored in liver, spleen and bone marrow.

Clinical conditions: Iron deficiency causes *hypochromic microcytic anemia*, characterized by low serum protein-bound iron (BI), poor tissue cytochromes and low hemoglobin levels.

Hemochromatosis is an excessive iron accumulation in tissues like spleen, liver, pancreas, gastrointestinal glands, heart, joints, pituitary and skin, caused by an excessive intestinal absorption of iron due to a genetic disorder. It occurs predominantly in men. Excessive hemosiderin deposits damage the tissues leading to a high serum iron, pancreatic fibrosis, diabetes mellitus, bronzing of skin (*bronzed diabetes*), degeneration of

cardiac muscles, congestive heart failure, hepatic fibrosis and hepatic failure.

Siderosis or hemosiderosis is an excessive iron accumulation in tissues due to secondary causes like habitual high iron-low phosphorus diets (e.g., in the African Bantu population), repeated blood transfusions, hereditary hemolytic anemias and thalassemias.

6.3 COPPER

Sources: Fish, oysters, liver, lentils, dry legumes and nuts.

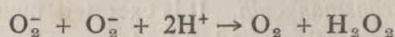
Daily dietary requirements: Adults : 2.5 mg; children : 0.5-2.5 mg.

Functions:

1. *Role in iron metabolism:* Copper helps in the utilization of iron for hemoglobin synthesis. *Ceruloplasmin* or *serum ferroxidase*, a blue copper-protein complex of blood plasma, catalyzes the oxidation of Fe^{2+} to Fe^{3+} ; this helps in the incorporation of iron in transferrin to facilitate mobilization of iron. A yellow copper-protein called *non-ceruloplasmin ferroxidase* may also participate in the oxidation of Fe^{2+} in human plasma. Deficiency of copper leads to *microcytic anemia* in sheep and pigs, living in copper-deficient regions. Human infants, kept on unsupplemented milk diet poor in copper, may develop a hypochromic microcytic anemia with low protein-bound iron in plasma, low serum copper and poor hemoglobin content; it does not respond to iron therapy, but a trace of copper, administered simultaneously with iron, promptly raises the blood reticulocyte count, hemoglobin concentration and serum protein-bound iron.

2. *Role in enzyme action:* Copper is present in the prosthetic groups of *metallo-protein oxidases* such as cytochrome oxidase, ascorbic acid oxidase, tyrosinase and uricase. *Cytochrome oxidase* activities are reduced in hepatic and cardiac mitochondria of copper-deficient chicken; a lack of hair pigment in

copper-deficient sheep indicates reduced activity of *tyrosinase* in melanin synthesis. *Serum ferroxidase* and *non-ceruloplasmin ferroxidase* have been mentioned above. Cytoplasmic *superoxide dismutase* of liver, nerve and red cells contains Cu^{2+} and Zn^{2+} and changes superoxide radicals to hydrogen peroxide :



3. *Maturation of elastin* : Copper helps to form insoluble elastin fibres by cross-linking soluble proelastin chains through the oxidation of some lysine sidechains of the latter into aldehydes. Proelastin rises significantly in copper-deficient pigs

4. *Constituent of hemocyanin* : Hemocyanin is a copper-protein respiratory pigment in the plasma of cephalopods, amphineurans and crustaceans.

Transport and fate : After intestinal absorption, Cu^{2+} enters the plasma and immediately binds to serum albumin. This copper-albumin complex reacts directly with diethyldithiocarbamate to produce a color (*direct-reacting copper*). Much of the direct-reacting copper is soon removed by the liver from plasma, combined with α -globulins and released into the plasma again. This fraction of serum copper must first be liberated by HCl from the protein before it can produce color with diethyldithiocarbamate (*indirect-reacting copper*). The ratio between direct and indirect reacting fractions of serum copper is usually 1 : 24. The indirect-reacting copper mainly exists as *ceruloplasmin*, a blue copper-glycoprotein carrying 4 Cu^{2+} and 4 Cu^+ ions per molecule or 0.35% copper by weight. 100 ml plasma may carry 25-30 mg ceruloplasmin holding 75-100 μg copper. Whole blood carries 100 μg Cu per 100 ml. Liver stores about 13-18% of the total body copper of 100-120 mg. Bile is the main channel for copper excretion.

Clinical conditions : In man, *Wilson's disease* (hepatolenticular degeneration) may occur due to an autosomal recessive genetic defect in either the synthesis of ceruloplasmin or the incorporation of Cu^{2+} into ceruloplasmin. The disease is characterized by low plasma copper, poor serum ferroxidase activity, low biliary copper, high urinary copper, high deposition of copper in the liver, pancreas, kidney, cornea and brain, and consequent symptoms such as abnormal muscular movements (like Parkinson's disease), diabetes mellitus, renal tubular damage, a visible brown ring (Kayser-Fleischer ring) at the margin of the cornea, dementia, hepatic cirrhosis and jaundice. The patient ultimately dies of hepatic failure.

Menke's disease (kinky hair syndrome) is characterized by skeletal malformations, mental retardation, immunological deficiency and defective thermoregulation. It results from an X-linked genetic defect in transporting the absorbed copper across the serosal membrane of intestinal mucosa cells.

Hypochromic microcytic anemia due to copper-deficiency in milk-fed infants has been described earlier.

6.9 ZINC

Sources : Liver, unmilled cereals, legumes, milk, maize, meat, spinach, lettuce, yeast, egg and oysters.

Daily requirements : Adult man/woman : 15 mg ; pregnant/lactating woman : 20-25 mg ; infants and children : 3-10 mg.

Deficiency : Zinc deficiency due to phytate-rich diet may cause poor growth, low erythrocyte carbonic anhydrase activity, alopecia and hypogonadism in man. In *acrodermatitis enteropathica* caused by a genetic failure of zinc absorption, poor growth, dermatitis, intestinal lesions, mental disorders, hypogonadism and eye defects are observed.

Functions :

1. *Role in enzyme action* : Zinc occurs in many metalloenzymes such as carboxypeptidase A, cytoplasmic superoxide dismutase, carbonic anhydrase and alcohol dehydrogenase.

2. *Role in vitamin A metabolism* : Zn^{2+} may stimulate the release of vitamin A from the liver into the blood. Moreover, Zn^{2+} -containing retinal reductase participates in the regeneration of rhodopsin in the eye following light exposure.

Distribution : Zinc is stored in the liver, skin and bones and is secreted in prostatic fluid, pancreatic juice, bile and epididymal fluid. Whole blood contains about 650-850 μg of zinc per 100 ml. It is present in the RBC mainly as carbonic anhydrase. About 120-140 μg of zinc occur in 100 ml of plasma, mostly in combination with serum albumin and transferrin. Adult body contains 2-3 g of Zn.

6.10 MANGANESE

Sources : Cereals, vegetables, fruits, nuts, tea and liver.

Daily requirements : Adult man/woman : 2.5-5 mg ; infants and children : 0.5-3 mg.

Functions :

1. *Enzyme action* : Mn^{2+} is either a cofactor or a constituent of many enzymes including arginase, mitochondrial superoxide dismutase, isocitrate dehydrogenase, cholinesterase, lipoprotein lipase, phosphotransferases, pyruvate carboxylase, acetyl-CoA carboxylase and glycosyltransferases.

2. *Role in animal reproduction* ; Mn^{2+} deficiency may produce sterility in cattle, and disturbances of estrous cycles, fetal resorption and sterility in sows.

3. *Proteoglycan synthesis* : Mn^{2+} plays some role in the synthesis and deposition of proteoglycans in many tissues including bones, by helping in glycosyltransferase activity. Mn^{2+} deficiency may lead to *perosis* (slipping

of gastrocnemius tendon) and bone deformities in chicks, and lameness in pigs.

4. *Porphyrin synthesis* : Mn^{2+} may help AmLev synthase in porphyrin synthesis.

Transport and fate : Manganese is stored in the liver. Blood contains 2-3 μg manganese per 100 ml. It is transported in the plasma mainly in combination with a β_1 -globulin called *transmanganin*. It is excreted mainly in bile and by colon. Adult body contains about 15-18 mg of manganese.

6.11 MOLYBDENUM

Adults and children require respectively about 0.5 and 0.05-0.3 mg Mo^{6+} per day ; cereals and dry legumes supply more than 50 μg per day. It is stored in the liver and kidneys, and excreted in the urine.

1. *Role in enzyme action* : It occurs in several non-heme iron flavoproteins ; e.g., xanthine oxidase of liver contains Mo^{6+} , S, Fe and flavin. It oxidizes xanthine to uric acid. Administration of molybdenum-antagonists (e.g., tungstate) to chicks lowers xanthine oxidase activity. Mo^{6+} also occurs in some hemoflavoproteins ; e.g., NADPH nitrate reductase of *Neurospora crassa*. Sulfite oxidase of human liver contains Mo^{6+} and heme, and oxidizes inorganic sulfites to sulfates. Nitrogenase of nitrogen-fixing bacteria contains Mo, Fe and S. Mo^{6+} of all these enzymes participates in *internal electron transfers* during oxidoreductions.

2. A trace of molybdenum may help in the *utilization of copper*. But high molybdenum intake produces microcytic anemia and low tissue copper in cattle and sheep.

6.12 COBALT

Adult human body contains only about 1-2 mg cobalt. It is carried in the plasma mainly in the cobalamin group of transcobalamin, is principally stored in the liver and excreted in the urine.

1. Cobalt may act as a *cofactor* for several enzymes like glycylglycine dipeptidase of intestinal juice. As a constituent of vitamin B₁₂, cobalt also occurs in cobamide coenzymes.

2. Cobalt helps in *erythropoiesis*. Cattle grazing in cobalt-deficient areas develop *macrocytic anemia* curable by either vitamin B₁₂ or cobalt sulfate. Continued administration of cobalt salts may greatly increase the number of circulating RBC (*polycythemia*). Cobalt is a constituent of vitamin B₁₂ which helps in RBC maturation. Cobalt may also increase the production of erythropoietin and may be a cofactor for AmLev synthase in heme synthesis.

6.13 SELENIUM

Daily requirements: Adults: 0.2 mg; infants and children: 0.02-0.1 mg.

Deficiency: Rats on low-selenium diet suffer from loss of hair, growth failure, hepatic necrosis and reproductive failure. Chicks on selenium-poor diet develop pancreatic fibrosis with reduced lipase secretion and a consequent failure of intestinal absorption of vitamin E. Although nutritional deficiency of vitamin E is primarily responsible for symptoms like muscular dystrophy in cows and lambs, hepatic necrosis in rats and swine, and exudative diathesis and cardiac myopathy in chicks, either selenium or vitamin E may prevent these symptoms. Significantly high incidences of cardiac dilatation, abnormal ECG and congestive cardiac failure have been observed among human residents of areas poor in soil selenium.

Functions:

1. *Glutathione peroxidase*: This seleno-protein occurs in erythrocytes, pulmonary cells and many other tissue cells and contains 4 selenium atoms. It uses glutathione as the donor of reducing equivalents (H⁺ and electron) for reducing H₂O₂ as well as fatty acid hydroperoxides (R-O-OH). The enzyme reduces the acyl hydroperoxides, formed

by oxidation of unsaturated fatty acids, into respective hydroxy acids (R-OH) with simultaneous oxidation of glutathione (GSH) to oxidized glutathione (GSSG): $R-O-OH + 2GSH \rightarrow R-OH + H_2O + GSSG$. The enzyme similarly reduces H₂O₂ to H₂O and prevents the peroxidative action of H₂O₂ on unsaturated fatty acids: $H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$. In these ways, this seleno-protein prevents peroxidative damages of membrane lipids, proteins or nucleic acids as also oxidation of hemoglobin.

2. *Tocopherol sparing action*: Selenium reduces the requirement for vitamin E in more than one way. (i) Se-containing glutathione peroxidase destroys acyl hydroperoxides, lowering the need for antioxidant action of vitamin E in preventing peroxidative damages. (ii) Se may maintain normal pancreatic function to promote vitamin E absorption. (iii) Se may probably help in retaining vitamin E in lipoproteins.

3. *Selenide-containing NHI proteins*: Se probably occurs as selenide at the active site of some non-heme iron proteins, located as integral proteins in microsomal and other cellular membranes (Fig. 7.44). Probably these seleno-proteins are associated with the mixed-function oxidase system of membranes.

Toxic effects: Selenium toxicity occurs in workers of paint, electronics, glass and ceramic industries. Ruminant animals grazing on selenium-rich soil may suffer from *alkali disease*. In this disease, selenium replaces sulfur in tissue and milk proteins and may inhibit sulphydryl enzymes.

6.14 IODINE

Sources: Iodized table salt, marine fishes, onion and iodate-enriched bread.

Daily requirements: Adult man: 140 µg; adolescent boy: 150 µg; adult woman: 100 µg; adolescent girl: 115 µg; pregnant woman: 125 µg; lactating woman: 150 µg; infants: 40 µg; children: 60-100 µg.

Functions : Inorganic iodides are actively collected into thyroid cells by an ATPase-dependent iodide transport mechanism (*iodide pump*) against an electrical gradient and a steep concentration gradient of about 1 : 10 to 1 : 25. Iodides are used in the synthesis of thyroid hormones. Dietary deficiency of iodine may produce thyroid enlargement (*iodine-deficiency goiter*) and hypothyroidism.

Distribution : About $\frac{1}{3}$ rd- $\frac{1}{5}$ th of the body iodine is collected in the thyroid which contains about 12 mg iodine. About 99.8% of the thyroidal iodine exists as organically bound iodine, chiefly in thyroglobulin of colloid. Each dl (100 ml) of plasma carries about 5-8 μ g of iodine bound to plasma proteins (*protein-bound iodine* or PBI) and only about 0.3 μ g as inorganic iodides. PBI rises and falls in hyperthyroidism and hypothyroidism respectively.

Excretion : 50-200 μ g of iodine may be excreted in 24 hours' urine as inorganic iodides and organic catabolites of thyroid hormones.

6.15 FLUORINE

Sources : Drinking water, tea, salmon, sardine and mackerel.

Daily requirements : Adult man/woman : 2-3 mg ; infants : 0.1-0.4 mg ; children : 0.5-2 mg.

Distribution : About 10-20 μ g of fluoride, mostly ionized, are present in 100 ml of blood. Fluorides occur in significant amounts in bones and teeth.

Functions :

1. **Tooth development and dental health :** Fluorine occurs in human teeth in traces. It helps in tooth development, normal maintenance and hardening of dental enamel and prevention of *dental caries*. Enamel destruction and dental caries are widespread where drinking water contains less than 0.5 parts of fluorine per million parts (0.5 ppm). Addition of very little fluoride in the drinking water or dental application of a very dilute

fluoride solution prevents or cures caries in developing teeth and maintains normal dental enamel. Probably, this *caristatic effect* of fluorine is due to its entry into the apatite salts of enamel. More than 1.2 ppm of fluorine in drinking water of infants and children may increase fluoride contents of the enamel and dentin, may reduce calcium deposition there, and may cause *mottling of enamel* in new permanent teeth. The mottled enamel is discolored, corroded, stratified and pitted. Still higher fluoride intakes cause abnormal tooth eruption and mottling of enamel in deciduous teeth also. Fluoride seems ineffective in curing caries and in mottling the enamel in fully formed adult teeth.

2. **Bone development :** Fluorine occurs in bones in traces. Catalytic amounts of fluorine are required for the conversion of calcium phosphates to *apatite* salts of bones and teeth. A trace of fluorine in food or water promotes bone development, increases Ca^{2+} and PO_4^{3-} retentions and prevents old-age osteoporosis. High fluoride intakes enhance abnormally the fluoride content, calcium deposition and density of bones.

High concentrations of fluorides are extremely toxic and inhibit Mg^{2+} -requiring enzymes.

6.16 CHROMIUM

Sources : Yeast, meat, cereals, milk, liver.

Daily requirements : Adult man or woman : 0.05-0.15 mg ; infants : 0.01-0.03 mg ; children : 0.02-0.1 mg.

Functions : In trace amounts, it may help to form a *glucose tolerance factor* which increases sugar tolerance, probably by increasing the sensitivity of the peripheral tissues to insulin. Experimental Cr^{3+} deficiency may lower the sugar tolerance and produce hyperglycemia in rats ; this is prevented or cured by Cr^{3+} administration. Cr^{3+} may also increase sugar tolerance in human patients suffering from protein-energy malnutrition.

7. VITAMINS AND COENZYMES

Animals, fed with a mixture of purified carbohydrates, lipids, proteins and minerals, develop deficiency symptoms which can be prevented or cured by certain foods containing small amounts of several widely divergent organic substances, called vitamins. Vitamins cannot be synthesized in the body in sufficient amounts, but are required in small amounts in the diet mainly for maintaining normal metabolic reactions. They are broadly classified as water-soluble and fat-soluble vitamins. *Water-soluble vitamins* include vitamin C and members of the vitamin B complex group, viz., thiamin, riboflavin, nicotinamide, pyridoxine, biotin, folates, pantothenic acid, cobalamins and inositol. *Fat-soluble vitamins* consist of vitamins A, D, E and K.

7.1 VITAMIN C

Sources : Citrus fruits like lemon, orange, pineapple, mango, litchi, grapes and strawberry, germinated legumes, guava, watermelon, green and red peppers, spinach and okra.

Daily requirements : Adult man or woman : 40 mg ; pregnant woman : 40 mg ; lactating woman : 80 mg ; infants and children : 20-40 mg.

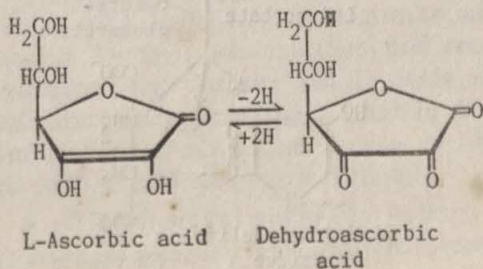


Fig. 7.1. L-Ascorbic acid and dehydroascorbic acid.

Chemistry : Vitamin C or L-ascorbic acid is the γ -lactone of a C_6 -sugar acid with a C_2 - C_3 double bond (Fig. 7.1). It forms white, sour, odourless crystals (m.p. 190°C). It dissolves in water to give a dextrorotatory acidic solution. Vitamin C is destroyed by aerobic oxidation and by heat, particularly in alkaline solutions. Vitamin C can be hydrogenated or halogenated at its double bond, forms metallic salts, acts as a reducing agent and is itself oxidized to dehydroascorbic acid. The latter may be reduced to ascorbic acid by H_2S and cysteine.

Synthesis : Most animals can synthesize vitamin C in the liver and adrenal cortex from glucuronic acid (Fig. 7.2). But man, apes, monkeys, red vented bulbul, Indian fruit bats and guineapig cannot synthesize L-gulonolactone oxidase of this pathway and consequently cannot synthesize ascorbic acid.

Fate : Adrenal cortex, adrenal medulla, pituitary, liver and corpus luteum store vitamin C. L-Ascorbate is oxidized to dehydroascorbate by cytochrome b_5 in animal tissues, and by a copper-enzyme called L-ascorbate oxidase in plants. Dehydroascorbate is reduced to L-ascorbate by a reductase and glutathione in animals. Vitamin C is excreted in urine as L-ascorbate and small amounts of dehydroascorbate and oxalate.

Deficiency ; In man and other animals incapable of ascorbate synthesis, dietary deficiency of vitamin C produces *scurvy*. Scurvy is characterized by defective formation and maintenance of intercellular substances like collagen and elastin of connective tissues, bone osteoid and tooth dentin. This may cause demineralization, weakness and easy fracture of bones, bleeding and spongy gums, loosening

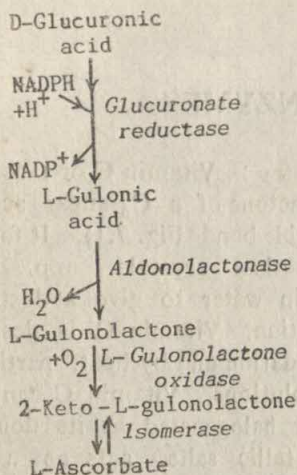


Fig. 7.2. Biosynthesis of L-ascorbate in nonprimates.

of teeth, delay in wound healing, increased fragility of blood vessels, spontaneous bruises and bleeding at joints. Anemia, fatigue, lassitude and urinary elimination of p-hydroxyphenylpyruvate and homogentisate may also occur. L-Ascorbate administration prevents or cures scurvy.

Functions: L-Ascorbate acts as the *antiscorbutic vitamin* and prevents scurvy by helping in the deposition and maintenance of intercellular substances in mesenchymal tissues. It acts as an electron-donor and reductant, and participates in several hydroxylation reactions as a cofactor.

1. Hydroxylation of proline and lysine: L-Ascorbate helps in post-translational hydroxylation of proline and lysine residues in immature collagen and elastin molecules to change them to mature collagens and elastins. Prolyl 4-hydroxylase is an Fe^{2+} -protein of microsomes of liver, muscle, heart, skin and granulation tissues. It utilizes an oxygen atom of molecular O_2 to hydroxylate the C^4 of a proline residue, peptide-bonded to the $\alpha\text{-NH}_2$ of a glycine residue in collagen (Fig. 7.3); α -ketoglutarate, a cosubstrate, is

simultaneously oxidized to succinate by the other oxygen atom of O_2 . L-Ascorbate functions as an electron-donor to maintain the reduced Fe^{2+} at the active site of the enzyme. Lysyl 5-hydroxylase similarly hydroxylates the C^5 of a lysine residue in collagen with the help of L-ascorbate. The latter helps in similar hydroxylations of proline residues in elastin molecules also. Failure of hydroxylation of collagens and elastins in scurvy leads to a rapid destruction of immature collagen, producing symptoms such as fragility of vessels, spongy bleeding gums, weakness of bones and delayed wound healing.

2. Hydroxylation of dopamine to norepinephrine: In adrenergic neurons and chromaffin cells of adrenal medulla, a Cu^{2+} -protein called dopamine β -hydroxylase utilizes L-ascorbate as an electron-donor in hydroxylating dopamine to norepinephrine (Fig. 7.4). Probably ascorbate donates two electrons at two different steps of this reaction, forming monodehydroascorbate as an intermediate.

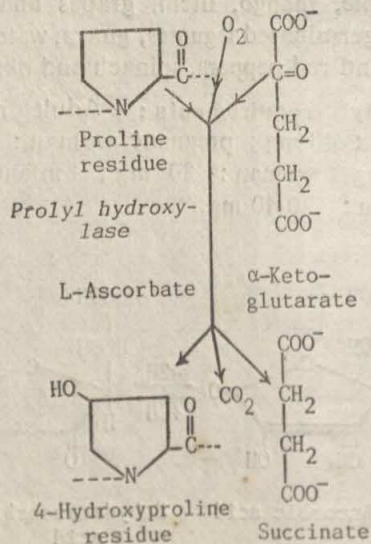


Fig. 7.3. Hydroxylation of a proline residue in collagen.

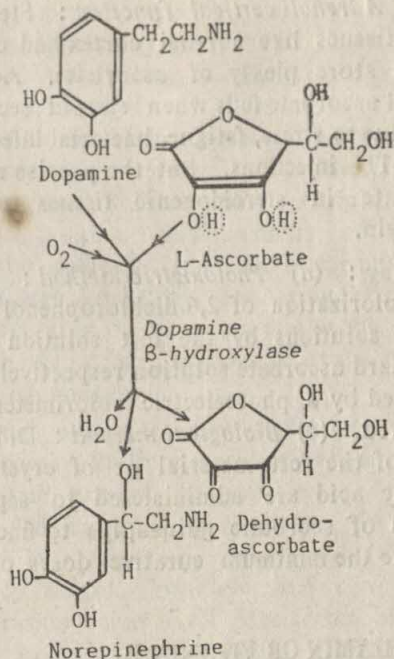


Fig. 7.4. Hydroxylation of dopamine.

3. *Hydroxylation reactions in carnitine synthesis*: L-Ascorbate probably acts as an electron-donor cofactor for γ -butyrobetaine hydroxylase which hydroxylates γ -butyrobetaine during its conversion to carnitine. L-Ascorbate probably also acts as a cofactor for ϵ -N-trimethyl-lysine hydroxylase which hydroxylates ϵ -N-trimethyl-lysine in its conversion to carnitine. These may explain the observed decline in carnitine content and in the activities of the two hydroxylases in tissues of scorbutic guineapigs. Carnitine helps to transfer fatty acids from cytoplasm to mitochondria for their beta oxidation and energy production; so, fatigue and lassitude may result in scorbutic men from a fall in tissue carnitine and a consequent decline in fatty acid oxidation for energy production.

4. *Oxidations in tyrosine catabolism*: In tyrosine catabolism, p-hydroxyphenylpyruvate hydroxylase (4-hydroxyphenylpyruvate dioxygenase) and homogentisate oxidase appear to

require L-ascorbate as an electron-donor. The first enzyme oxidatively decarboxylates the pyruvate sidechain of p-hydroxyphenylpyruvate, shifts the resulting acetate sidechain to the ortho position and hydroxylates the carbon at the para position vacated by the acetate sidechain (Fig. 7.5). The product homogentisate is oxidized by the second enzyme to maleylacetoacetate. The postulated role of L-ascorbate as a cofactor for these two enzymes may explain the rise in serum tyrosine and the urinary elimination of p-hydroxyphenylpyruvate and homogentisate in scurvy; these symptoms are reduced on ascorbate administration.

5. *Amidation of peptidylglycine*: The electron transport function of L-ascorbate is believed to enhance manifold the *in vitro* activity of a peptidylglycine α -amidating monooxygenase. This Cu²⁺-requiring enzyme removes two hydrogens from the α -C and the α -amino-N of the C-terminal glycine residue of precursor peptides destined to form vasopressin, oxytocin, gastrin, cholecystokinin, vasoactive intestinal peptide, calcitonin, CRH, GHRF, etc. (Fig. 7.6). A double bond is thus

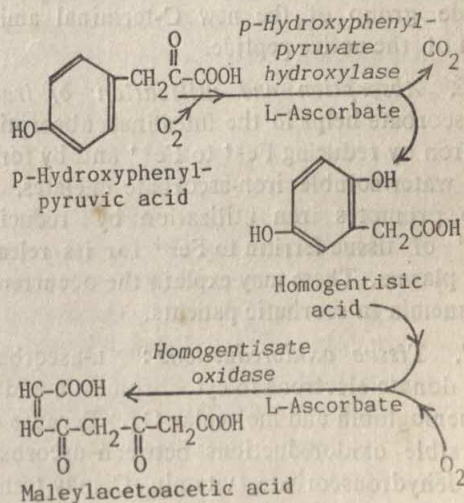


Fig. 7.5. Ascorbate in metabolism of p-hydroxyphenylpyruvate.

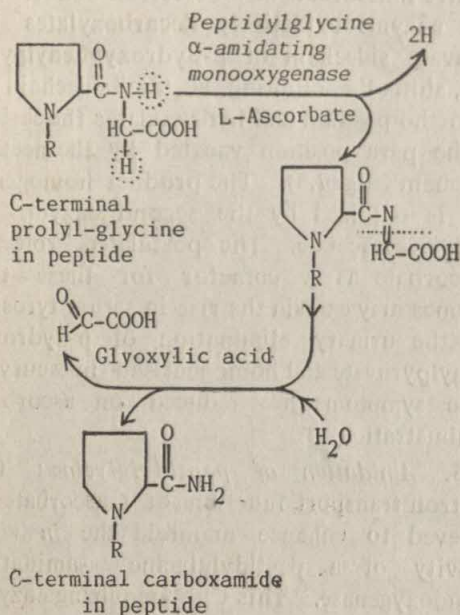


Fig. 7.6. Amidation of peptidylglycine.

formed between the α-C and the α-N of glycine residue. This double bond is then hydrolytically split to release the glycine as glyoxylate, leaving its nitrogen as the carboxamide group of the new C-terminal amino acid of the active peptide.

6. Absorption and utilization of iron: L-Ascorbate helps in the intestinal absorption of iron by reducing Fe^{3+} to Fe^{2+} and by forming water-soluble iron-ascorbate chelates. It also promotes iron utilization by reducing Fe^{3+} of tissue ferritin to Fe^{2+} for its release into plasma. These may explain the occurrence of anemia in scorbutic patients.

7. Tissue oxidoreductions: L-ascorbate may donate electrons to cytochromes a and c, methemoglobin and molecular O_2 . Because of reversible oxidoreductions between ascorbate and dehydroascorbate, vitamin C may transport electrons from some substrates like NADH, phenolic amino acids, glutathione and flavoproteins to cytochromes c and a.

8. Adrenal cortical function: Steroidogenic tissues like adrenal cortex and corpus luteum store plenty of ascorbate. Adrenal cortical ascorbate falls when steroid secretion rises due to stress, fatigue, bacterial infections or ACTH injections. But the precise role of ascorbate in steroidogenic tissues remains uncertain.

Assay: (a) *Photometric method:* Rates of decolorization of 2,6-dichlorophenol indophenol solutions by the test solution and a standard ascorbate solution respectively, are measured by a photoelectric colorimeter and compared. (b) *Biological method:* Different doses of the test material or of crystalline ascorbic acid are administered to separate batches of scorbutic guineapigs to find and compare the minimum curative doses of the two.

7.2 THIAMIN OR VITAMIN B₁

Sources: Yeast, liver, outer layers of cereal grains, peanuts, dry peas and beans, nuts, eggs, lean meat, lentils and soyabeans.

Daily requirements: Adult man: 1.4 mg; adult woman: 1.0 mg; pregnant woman: 1.3 mg; lactating woman: 1.5 mg; children and infants: 0.6-1.3 mg. The requirement rises with a rise in calorie intake.

Chemistry: Thiamin ($\text{C}_{12}\text{H}_{17}\text{N}_4\text{OS}$) is composed of 4-methyl-5-hydroxyethylthiazole and 2,5-dimethyl-6-aminopyrimidine (Fig. 7.7). Thiamin hemihydrate forms white, needle-shaped, water-soluble crystals (m.p. 248-250°C) with a characteristic odour. It is generally destroyed by heat, reducing agents or oxidizing agents.

Deficiency: Thiamin deficiency may be produced in rats either by B_1 -deficient diets or by administering *thiamin antagonists* (antivitamins B_1) like pyriethiamin. This causes *polyneuritis* with loss of appetite, poor growth, rise in blood pyruvate and pentose, inflammation and degeneration of limb nerves. In

pigeons made polyneuritic by B_1 -deficient diets, rise in pyruvate and lactate levels in blood and brain, inflammation of peripheral nerves, paralysis of limbs and head-retraction (opisthotonus) are observed. Foxes, thriving on raw fish, may develop B_1 -deficiency due to thiaminase of fish muscles; symptoms include brain lesions, inflammations of peripheral nerves, defective limb movements and paralysis (*Chastek paralysis*). Sea-faring fishermen may suffer from B_1 -deficiency due to the consumption of raw fish; cooking destroys thiaminase in fish muscle.

In man, thiamin deficiency produces *beriberi*. Beriberi is widespread amongst low-income groups of south-east Asia where B_1 -deficient polished rice forms the main item of diet. Anorexia, nausea, vomiting, loss of body weight, lassitude and rise in blood pyruvate occur in all three forms of beriberi.

(i) In *dry or neuritic beriberi*, inflammation of peripheral nerves may lead to hyperaesthesia, pain, paralysis and wasting of limb muscles, enlarged heart, inflammation of ophthalmic nerve and blindness; there may also be lesions in the ventral horn of spinal cord, inflammation of dorsal root ganglia, loss of deep reflexes, mental confusions and anxiety. (ii) In *wet or edematous beriberi*, extensive edema in the extremities and heart, congestive heart failure and cardiac enlargements are seen. (iii) *Wernicke-Korsakoff syndrome* is characterized by lesions and hemorrhages near the third cerebral ventricle, paralysis of eye movements, insomnia, abnormal gait and stance, depression, deranged mental functions, disorientation, extreme anxiety, severe loss of memory and mental confusions. This syndrome is produced by B_1 -deficiency in patients genetically deficient in transketolase activity.

Hypervitaminosis: It has been reported that prolonged high intake of thiamin may produce headache, insomnia, tachycardia, irritability and anaphylactic reactions in human patients.

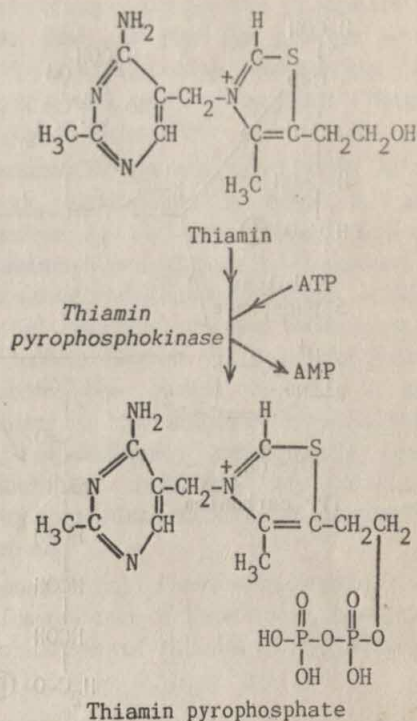


Fig. 7.7. Biosynthesis of TPP.

Functions: Most of the vitamin is changed to thiamin pyrophosphate (TPP) in the liver and intestinal mucosa with the help of ATP and thiamin pyrophosphokinase (Fig. 7.7).

1. TPP is a coenzyme for the transfer of activated aldehyde groups to specific acceptors. In this way, it acts as a coenzyme of (i) pyruvate dehydrogenase and (ii) α -ketoglutarate dehydrogenase for oxidative decarboxylations of respectively pyruvate and α -ketoglutarate in aerobic catabolism of pyruvate, and (iii) transketolase for α -ketol formation in the pentose phosphate pathway.

In all these three cases, a very reactive carbanion (C^-) is first formed by the ionization of the carbon atom linking the nitrogen and sulfur of the thiazole ring of enzyme-bound TPP. The carbanion binds with the

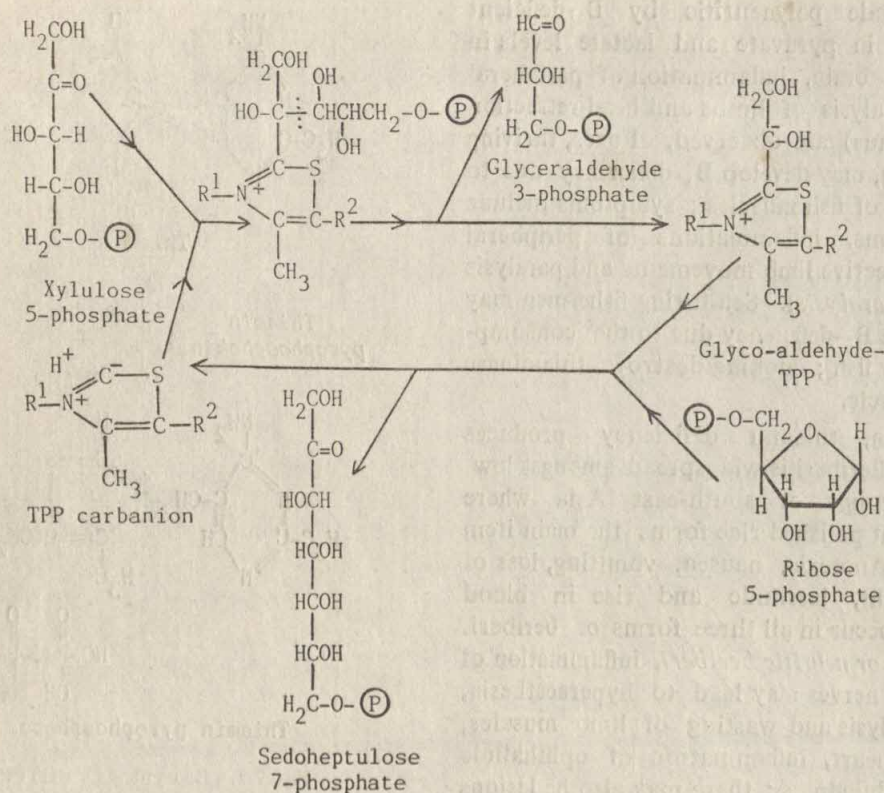


Fig. 7.8. TPP in transketolase action.

carbonyl (C=O) group of the substrate, viz., pyruvate, α -ketoglutarate or xylulose 5-phosphate for respectively pyruvate dehydrogenase (PDH), α -ketoglutarate dehydrogenase (α -KGD) and transketolase. The substrate-TPP addition compound then loses CO₂ in case of the first two enzymes, but a molecule of glyceraldehyde 3-phosphate in case of transketolase (Figs. 7.8 and 7.9). Hydroxyethyl TPP, α -hydroxy- β -carboxypropyl-TPP and glycoaldehyde-TPP are consequently formed in the three cases respectively.

In case of transketolase, the glycoaldehyde group is transferred from TPP to another aldose, either ribose 5-phosphate or erythrose 4-phosphate (Fig. 7.8). The resulting product is a new ketose; the latter is either sedoheptulose 7-phosphate or fructose 6-phosphate

according as the acceptor is ribose 5-phosphate or erythrose 4-phosphate.

But in case of PDH and α -KGD, the hydroxyethyl or α -hydroxy- β -carboxypropyl group is oxidized by the transfer of reducing equivalents (H⁺ and electron) to α -lipoic acid. The oxidized product, viz., acetyl or succinyl group respectively, is simultaneously transferred from TPP to the reduced acceptor dihydrolipoate (Fig. 7.9). The latter in turn transfers the oxidized group to coenzyme A to form acetyl-CoA or succinyl-CoA respectively.

2. In a manner identical with that for PDH and α -KGD, TPP functions also as a coenzyme for a mitochondrial *branched-chain α -keto acid decarboxylase*. This enzyme catalyzes oxidative decarboxylations of

branched-chain α -keto acids formed in the catabolism of valine, leucine and isoleucine. TPP binds with and decarboxylates those branched-chain α -keto acids, and transfers the resulting activated aldehyde groups to α -lipoic acid.

3. TPP acts as the coenzyme (*cocarcboxylase*) of pyruvate decarboxylase in yeast for the non-oxidative decarboxylation of pyruvate to acetaldehyde by a mechanism resembling the decarboxylation of pyruvate by mammalian pyruvate dehydrogenase.

As TPP helps in pyruvate oxidation, pyruvate and lactate levels rise in the blood, tissues and urine in beriberi, causing vasodilatation and edema. As TPP is also a coenzyme for the metabolism of branched-chain α -keto acids formed from valine, leucine and isoleucine, those α -keto acids also rise in blood and urine in beriberi. As TPP is needed for transketolase activity, decline in that activity

in beriberi depresses pentose phosphate metabolism and elevates the pentose levels of tissues and erythrocytes. Estimation of *erythrocyte transketolase activity* has a diagnostic value for B_1 deficiency.

Because of the role of thiamin in carbohydrate metabolism, it helps in *glucose utilization by the nerve tissue*. So, energy production from glucose is depressed in the nerve tissue and heart in beriberi, leading to neuronal inflammations and lesions, lassitude and cardiac failure. In Wernicke-Korsakoff syndrome, the patient inherits a genetic reduction in the affinity of transketolase for TPP; B_1 -deficiency consequently causes a considerable reduction in transketolase activity to produce the severe neuropsychiatric syndrome.

Assay : (a) *Fluorometric methods* : The blue fluorescence of thiochrome, produced by the oxidation of thiamin with alkaline potas-

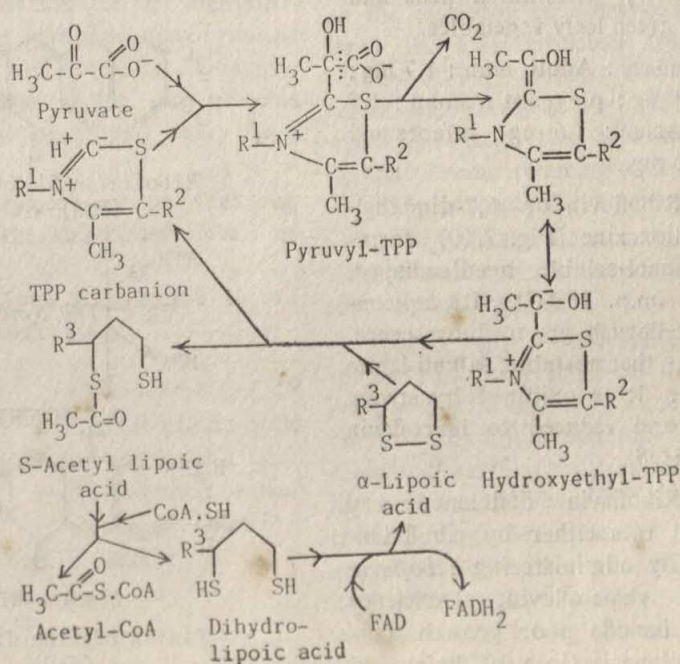


Fig. 7.9. TPP in pyruvate dehydrogenase action.

sium ferricyanide, is measured in a fluorometer or spectrophotometer for estimating thiamin in a test material. The basis for another fluorometric method is the production of a fluorescent substance from thiamin by reaction with cyanogen bromide. (b) *Colorimetric method*: Thiamin extracted from a test material is treated with diazotized p-aminoacetophenone to give a red pigment whose color intensity is compared by a photoelectric colorimeter with that produced in a standard thiamin solution. (c) *Microbiological method*: The basis for this method is the stimulation of yeast fermentation in presence of thiamin. (d) *Biological method*: Growth responses or durations of cure of thiamin-deficient rats on administration of the test material are compared with similar effects of pure thiamin preparations.

7.3 RIBOFLAVIN OR VITAMIN B₂

Sources: Yeast, marmite, liver, milk, cheese, wheat germ, germinated peas and legumes, eggs and green leafy vegetables.

Daily requirements: Adult man: 1.7 mg; adult woman: 1.3 mg; pregnant woman: 1.5 mg; lactating woman: 1.6 mg; infants and children: 0.7-1.6 mg.

Chemistry: Riboflavin or 6,7-dimethyl-9-D-1'-ribityl-isoalloxazine (Fig. 7.10) forms water-soluble, ethanol-soluble, needle-shaped, orange crystals (m.p. 282°C). Its aqueous solution emits a yellowish green fluorescence. It is acid-stable, thermostable, alkali-labile and photolabile. It is oxidized by strong oxidizing agents, and reduced to leucoflavin by thiosulfate or H₂S.

Deficiency: Riboflavin deficiency may be produced in rats either by riboflavin-deficient diets or by administering *riboflavin antagonists* like galactoflavin. Deficiency symptoms in rats include poor growth, dermatitis, cataract, alopecia (loss of hair) and reproductive failure.

In man, riboflavin deficiency produces (i) *cheilosis* or angular fissures in the lips, (ii) *glossitis* or a magenta-colored inflammation of the tongue, (iii) *seborrheic dermatitis* with shark-like thickening and atrophy of skin at nasolabial folds, ear, forehead and scrotum, and (iv) *corneal vascularisation*. Continued intake of the drug chlorpromazine

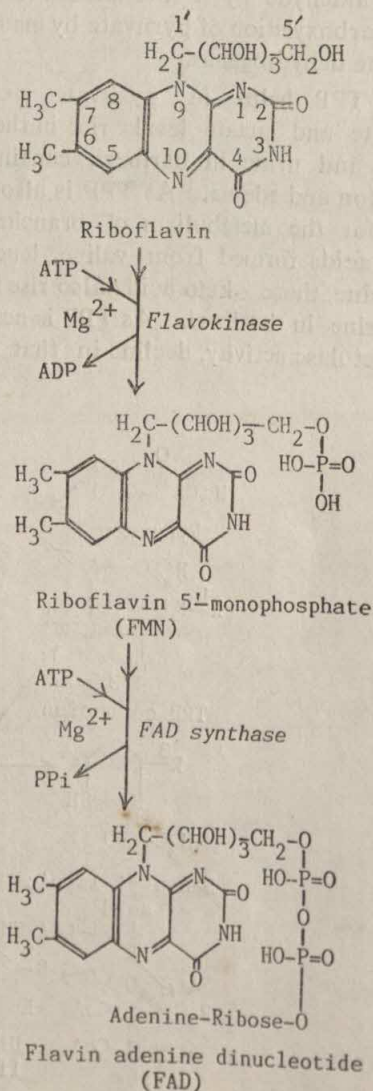


Fig. 7.10. Biosynthesis of flavin coenzymes.

may produce riboflavin deficiency by competitively inhibiting the conversion of riboflavin to its active derivative FMN. Phototherapy of hyperbilirubinemia may cause riboflavin deficiency by photolyzing the circulating riboflavin.

Functions :

1. *Flavin mononucleotide (FMN)* and *flavin adenine dinucleotide (FAD)* are the coenzymes formed from riboflavin in liver, intestinal mucosa and other tissues. FMN or riboflavin 5'-monophosphate is formed by the phosphorylation of riboflavin by ATP and flavokinase; FAD is produced by the transfer of an adenylate group from ATP to FMN by FAD synthase (Fig. 7.10). These coenzymes remain tightly bound to respective apoenzymes to form *flavoprotein oxidoreductases* and do not dissociate from them easily (Table 7.1).

Some flavoprotein enzymes are *metallo-flavoproteins* containing metals like iron and molybdenum in addition to FMN or FAD; e.g., xanthine oxidase and NADH dehydrogenase. Some again are *hemoflavoproteins* containing both heme and flavin, e.g., L-lactate dehydrogenase of yeast. Some flavoproteins such as sulfite reductase (yeast) carry both FMN and FAD.

Flavoprotein enzymes act as oxidizing enzymes catalyzing the dehydrogenation of

TABLE 7.1. *Some flavoprotein oxidoreductases.*

Enzymes containing FAD	Enzymes containing FMN
Succinate dehydrogenase	NADH dehydrogenase
Acyl-CoA dehydrogenase	Cytochrome b_2
D-Amino acid oxidase	L-Amino acid oxidase
Xanthine oxidase	Warburg's yellow enzyme
α -Glycerophosphate dehydrogenase (mitochondrion)	
NADPH-cytochrome P-450 reductase	
Dihydrolipoyl dehydrogenase	

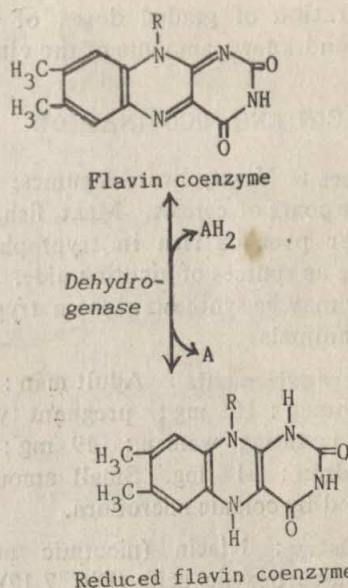


Fig. 7.11. Action of flavin coenzyme. AH_2 : reduced substrate. A: oxidized substrate.

their substrates. During catalytic activity, the isoalloxazine ring of the flavin functions as the acceptor or donor of reducing equivalents (H^+ and electron) from or to the substrate (Fig. 7.11). The reduced flavin coenzyme, formed by receiving reducing equivalents from the substrate, is reoxidized by transferring reducing equivalents to molecular O_2 or some other acceptor.

2. Riboflavin helps in the proper maintenance and health of ectodermal tissues like skin and oral mucosa.

Assay : (a) *Fluorometric method* : The intensity of fluorescence of the test sample is compared with that of a standard solution of riboflavin by a fluorophotometer. (b) *Microbiological method* : Rises in lactic acid production by *Lactobacillus caesi*, on administration of graded doses of the test material, are compared with similar effects produced by known amounts of the vitamin. (c) *Biological method* : The vitamin is estimated in a test material by comparing growth responses of riboflavin-deficient chicks or rats, on

administration of graded doses of the test material and known amounts of the vitamin.

7.4 NIACIN AND NICOTINAMIDE

Sources : Yeast, liver, legumes, peanuts and outer coats of cereals. Meat, fish, prawn and other proteins rich in tryptophan may also serve as sources of nicotinamide, because the latter may be synthesized from tryptophan in many animals.

Daily requirements : Adult man : 19 mg ; adult woman : 15 mg ; pregnant woman : 17 mg ; lactating woman : 19 mg ; infants and children : 8-14 mg. Small amounts are synthesized by colonic microflora.

Chemistry : Niacin (nicotinic acid) or pyridine 3-carboxylic acid (Fig. 7.12) forms white needle-shaped crystals (m.p. 236°C) and is thermostable, photostable, soluble in water, glycerol and ethanol. Niacinamide or nicotinamide or pyridine 3-carboxylic acid amide (m.p. 131°C) is a water-soluble solid. The COOH group of niacin reacts with alkalis to form salts while the pyridine-N forms salts with mineral acids.

Deficiency : Deficiency of nicotinamide produces *pellagra* in man. This disease is widespread amongst people subsisting mainly on maize or 'jowar' (*Sorghum vulgare*) ; these grains contain little tryptophan and carry most of the nicotinamide in the outer coats which are lost during milling. Besides, high leucine-content of 'jowar' depresses nicotinamide biosynthesis.

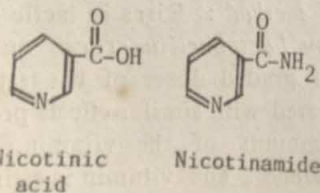


Fig. 7.12. Nicotinic acid and nicotinamide.

Nicotinamide antagonists like 6-amino-nicotinamide and 3-acetylpyridine may also produce nicotinamide deficiency.

Symptoms of pellagra include (i) *glossitis* or inflammation of tongue making the tongue red, swollen, smooth, ulcerated and atrophic, (ii) *stomatitis* or inflammation and fissures of lips, (iii) *exfoliative dermatitis* characterized by red, itchy, dry, thickened, pigmented, vesiculated and desquamated skin in hands, feet, axilla, wrists and knees, (iv) *diarrhoea* with intestinal lesions and vomiting, and (v) *dementia* with cerebral and spinal lesions, irritability and anxiety. Unless treated with niacin or nicotinamide, the disease may prove fatal.

In dogs, niacin deficiency produces *black-tongue* with stomatitis, glossitis, diarrhoea, gastrointestinal lesions and fatty liver.

Hypervitaminosis : Excessive nicotinamide feeding produces fatty liver in rats due to a shortage of methyl groups for choline synthesis as nicotinamide detoxication uses up methyl groups. High doses of niacin (but not of nicotinamide) produces cutaneous vasodilatation, flushing syndrome, gastrointestinal symptoms and pruritis in man.

Synthesis : Some nicotinamide is synthesized in the liver from tryptophan. L-Tryptophan is oxidized by tryptophan 2,3-dioxygenase, a heme enzyme, to form N-formylkynurenine which is deformylated by kynurenine formylase to kynurenine (Fig. 7.13). The latter is hydroxylated to 3-hydroxykynurenine by kynurenine 3-hydroxylase, NADPH and molecular O₂. 3-hydroxykynurenine is cleaved by kynureninase and pyridoxal phosphate to give alanine and 3-hydroxyanthranilate. The latter is oxidized by a dioxygenase to α -amino- β -carboxymuconic acid semialdehyde which cyclizes to quinolinic acid. Quinolinic acid receives a phosphoribosyl group from 5-phosphoribosyl 1-pyrophosphate (PP-ribose-P) and also gets decarboxylated into nicotinate mononucleotide (NMN). NMN may also

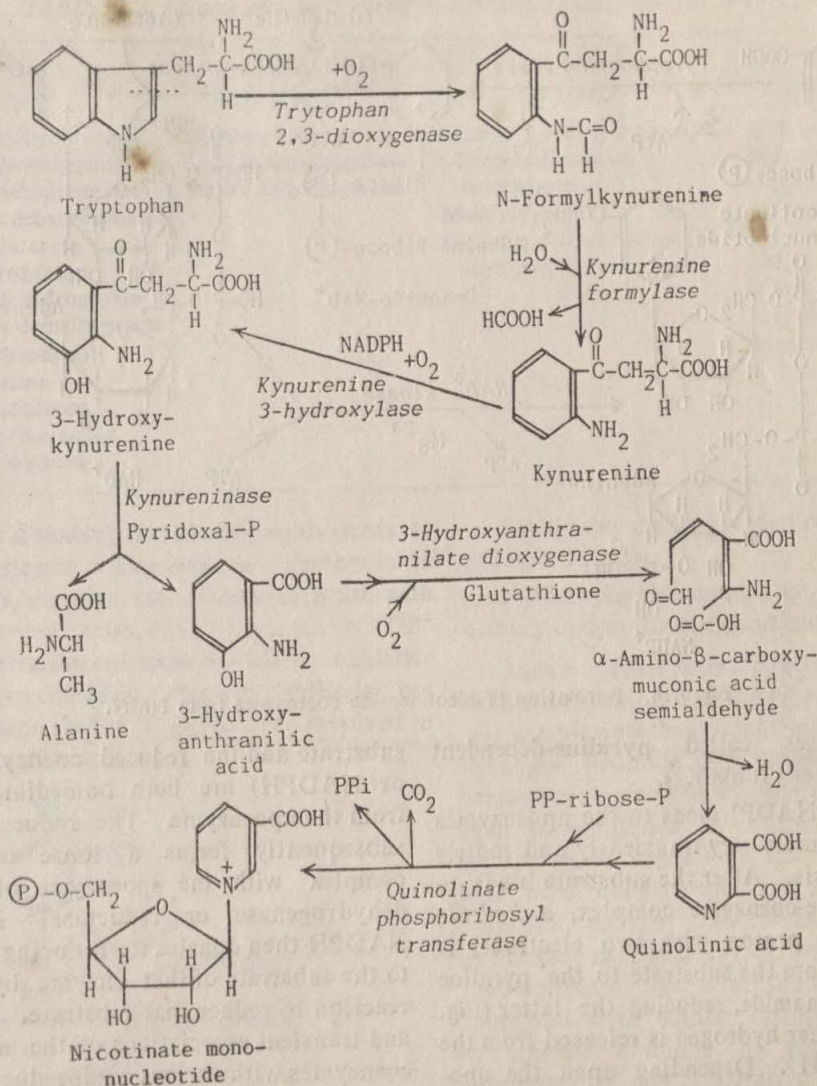


Fig. 7.13. Biosynthesis of nicotinate mononucleotide.

be formed from dietary nicotinic acid by the transfer of a phosphoribosyl group from PP-ribose-P.

NMN receives an adenylate group (AMP) from ATP under the action of an adenylate transferase and pyrophosphorylase to form desamido-NAD⁺ (Fig. 7.14). The latter receives an amide group from glutamine by transamidation to produce nicotinamide

adenine dinucleotide (NAD⁺). NAD⁺ may be phosphorylated by NAD⁺ kinase and ATP to give NADP⁺ (NAD⁺ phosphate).

Functions: Nicotinic acid and nicotinamide prevent and cure pellagra.

The nicotinamide nucleotides, viz., NAD⁺, NADP⁺ and their reduced forms (NADH and NADPH), function as coenzymes for many

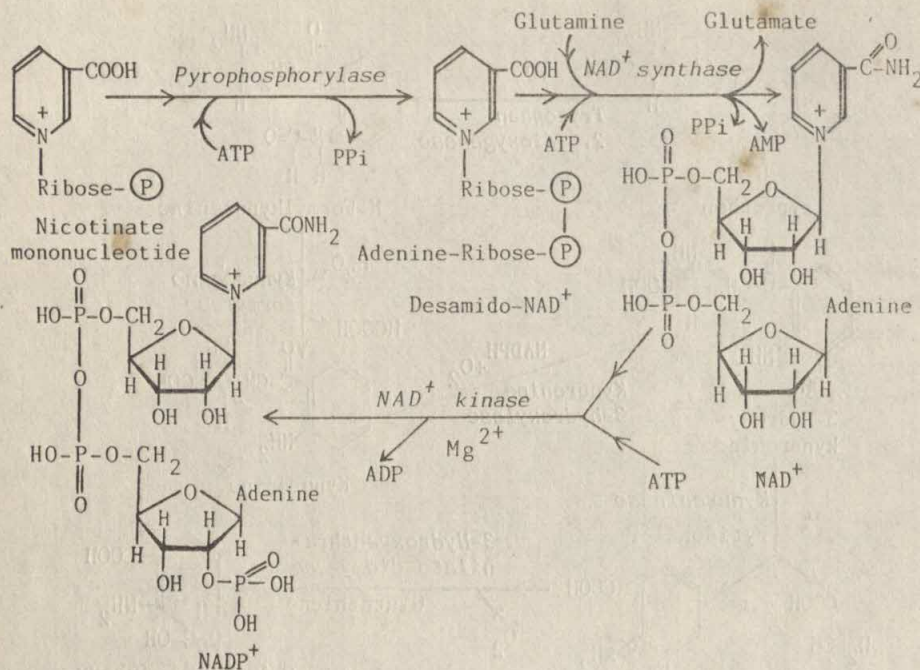


Fig. 7.14. Formation of nicotinamide coenzymes from NMN.

oxidoreductases called pyridine-dependent dehydrogenases (Table 7.2).

NAD^+ or NADP^+ binds to the apoenzymes of dehydrogenases very transiently and loosely during catalysis. After the substrate binds to the apoenzyme-coenzyme complex, a *hydride ion* (H^- or a proton with two electrons) is transferred from the substrate to the pyridine ring of nicotinamide, reducing the latter (Fig. 7.15); the other hydrogen is released from the substrate as H^+ . Depending upon the apoenzyme, the nicotinamide coenzyme binds the accepted hydrogen on a specific side of the plane of its pyridine ring, producing either A or B form of NADH (or NADPH). For example, alcohol dehydrogenase is *A-stereospecific*; it transfers hydrogen from its substrate to NAD^+ , producing the A form of NADH. But glyceraldehyde 3-phosphate dehydrogenase is *B-stereospecific*; it transfers hydrogen from its substrate to NAD^+ , producing the B form of NADH. The oxidized

substrate and the reduced coenzyme (NADH or NADPH) are both immediately released from the apoenzyme. The reduced coenzyme subsequently forms a loose and transient complex with the apoenzyme of some other dehydrogenase or reductase. NADH or NADPH then donates its reducing equivalents to the substrate of that enzyme by a reverse reaction to reduce that substrate. Such loose and transient associations of the nicotinamide coenzymes with various oxidoreductases enable them to transfer electrons and H^+ from substrate to substrate. Moreover, the relative stability of NADH or NADPH, owing to the multiple resonance forms of the reduced pyridine ring, makes the latter an effective *electron sink* for many oxidoreductions.

NADH gets reoxidized to NAD^+ , mostly by transferring its reducing equivalents (H^+ and electron) to the mitochondrial electron transport chain. This helps in energy production. But NADPH is reoxidized to NADP^+ ,

TABLE 7.2. Some enzymes using nicotinamide nucleotides as coenzymes.

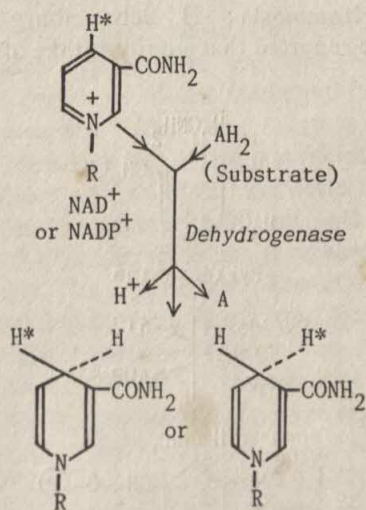
Enzymes using NAD^+	Enzymes using $NADH$	Enzymes using $NADP^+$	Enzymes using $NADPH$
Glyceraldehyde 3-P dehydrogenase	Cystine reductase	Glucose 6-P dehydrogenase	HMG-CoA reductase
Lactate dehydrogenase	Lactate dehydrogenase	6-Phosphogluconate dehydrogenase	Squalene synthase
Pyruvate dehydrogenase	Malate dehydrogenase	Malic enzyme	Glutathione reductase
α -Ketoglutarate dehydrogenase		Isocitrate dehydrogenase (cytoplasm)	β -Ketoacyl reductase
Malate dehydrogenase			Enoyl reductase
Isocitrate dehydrogenase (mitochondria)			Aldose reductase
UDP-glucose dehydrogenase			Dihydrofolate reductase
β -Hydroxyacyl-CoA dehydrogenase			Fatty acid desaturase

mostly by donating its reducing equivalents to other substrates during reductive syntheses of fatty acids, steroids, etc. Commensurate with these divergent roles, NAD^+ exceeds $NADP^+$ in concentration in muscles where oxidative processes overshadow reductive synthesis, but $NADP^+$ exceeds NAD^+ in the liver involved in many reductive syntheses.

Niacin and nicotinamide prevent and cure pellagra in man.

N-Methylnicotinamide is the principal urinary endproduct of nicotinamide.

Assay : (a) *Photoelectric method* : Niacin derivatives are all hydrolyzed to niacin in the test sample and the products are treated with cyanogen bromide and aniline (or p-aminoacetophenone) to give a yellow pigment whose light transmittance is compared by a photoelectric photometer with that of a standard solution of niacin, similarly treated. (b) *Microbiological method* : Increases in lactic acid production by *Lactobacillus arabinosus* due to the stimulating effects of the test material are compared with similar changes on addition of known amounts of the vitamin. (c) *Biological method* : Curative effect of niacin and its derivatives on niacin-deficient dogs, suffering from blacktongue, forms the basis of assay.



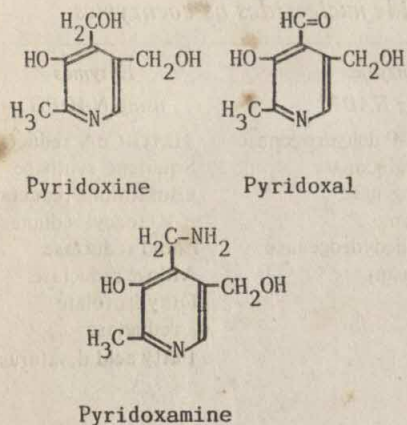
'A' form of
NADH or NADPH 'B' form of
NADH or NADPH

Fig. 7.15. Actions of nicotinamide coenzymes.

7.5 PYRIDOXINE OR VITAMIN B₆

Pyridoxine, pyridoxal and pyridoxamine have similar biological effects and are grouped together under vitamin B₆.

Sources : Yeast, liver, cereal grains, egg-yolk and meat.

Fig. 7.16. Compounds with vitamin B₆ activity.

Daily requirements : Adults : 2 mg ; pregnant or lactating woman : 2.5 mg ; infants and children : 0.3-1.6 mg.

Chemistry : Pyridoxine or 2-methyl-3-hydroxy-4,5-hydroxymethylpyridine is a weak, thermostable and photolabile base. It forms salts with mineral acids and gives a violet color with FeCl₃. It forms white, odourless, water-soluble and ethanol-soluble crystals (m.p. 160°C). Pyridoxal and pyridoxamine carry a CHO group and a CH₂NH₂ group

respectively, in place of 4-hydroxymethyl group of pyridoxine (Fig. 7.16). Both are photolabile and thermolabile.

Deficiency : In rats, B₆ deficiency produces poor growth, convulsions, urinary excretion of xanthurenic acid (an abnormal metabolite of tryptophan), microcytic hypochromic anemia, and a symmetrical edematous dermatitis (*acrodynia*) on both sides of head, tail and front paws. Pyridoxine deficiency in human infants may cause epileptiform convulsive seizures, abnormal EEG, microcytic anemia and urinary excretion of xanthurenate, homocystine and cystathionine. Administration of *pyridoxine antagonists* like methoxypyridoxine and isoniazide to human subjects produces seborrheic dermatitis, polyneuritis, subnormal concentrations of essential fatty acids in plasma, oxaluria, urinary excretion of xanthurenate, lymphopenia and sometimes, symptoms of pellagra. Isoniazide, an anti-tubercular drug, binds with pyridoxal to form pyridoxal hydrazone which is rapidly eliminated in the urine, producing B₆ deficiency.

Hypervitaminosis : H. Schaumburg and others have reported that a daily intake of 2 g

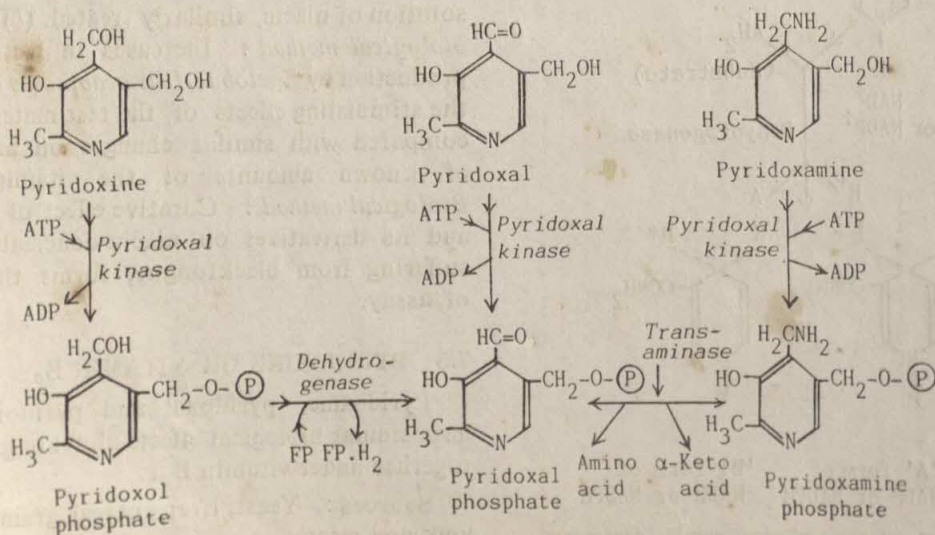


Fig. 7.17. Biosynthesis of pyridoxal phosphate.

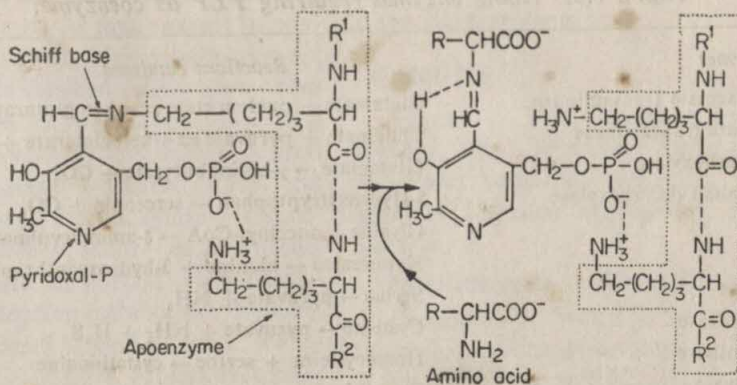


Fig. 7.18. Replacement of apoenzyme-PLP Schiff base by substrate-PLP Schiff base.

or higher of vitamin B₆ produces severe neuronal damages, a loss of cutaneous sensations on limbs and an inability to walk. On discontinuation of B₆ intake, recovery takes place very slowly and is frequently incomplete. Excessive intake of vitamin B₆ by pregnant mothers may subsequently result in convulsions in their new-born infants.

Functions :

1. Pyridoxal 5-phosphate (PLP) and pyridoxamine 5-phosphate are the biologically active forms of this vitamin and function as the coenzymes for many enzymes of *amino acid metabolism*. Pyridoxal kinase phosphorylates pyridoxal to PLP with the help of ATP in the liver, brain and other tissues (Fig. 7.17). Pyridoxine is phosphorylated similarly by pyridoxal kinase to form pyridoxol phosphate which is oxidized to pyridoxal phosphate by a flavoprotein (FP) dehydrogenase (Fig 7.17). Pyridoxal kinase may also phosphorylate pyridoxamine to pyridoxamine phosphate. The latter is interconvertible with PLP through the removal or addition of an amide group on the C⁴-sidechain.

PLP remains bound to its apoenzyme by (i) an ionic bond between its anionic phosphate group and a cationic NH₃⁺ of a basic amino acid sidechain of the apoenzyme, and (ii) a

Schiff base linkage between its C⁴-aldehyde group and an ε-NH₂ of a lysine residue of the apoenzyme (Fig. 7.18). Of the two bonds, only the C⁴-Schiff base linkage opens up during catalysis and a new Schiff base linkage is formed, binding the α-NH₂ of the substrate amino acid with the C⁴-aldehyde group of the enzyme-bound PLP. This helps labilize specific covalent bonds in the bound α-amino acid, depending on the apoenzyme, and accordingly catalyzes transamination, decarboxylation, desulfhydration or dehydration of the amino acid (Table 7.3).

As the coenzyme for *transaminases* like glutamate pyruvate transaminase and glutamate oxaloacetate transaminase of liver and kidney, PLP receives the NH₂ group from an amino acid to change the latter to an α-keto acid and itself to form pyridoxamine phosphate. The latter is reconverted to PLP by donating the NH₂ group to another α-keto acid which thereby changes to a new α-amino acid (Fig. 7.19). At each step, an enzyme-bound Schiff base intermediate is formed by the binding of the coenzyme with the corresponding substrate.

Many of the symptoms of B₆-deficiency can be explained on the basis of the coenzyme activities of PLP (Table 7.3). Microcytic anemia, accompanying B₆-deficiency, may

TABLE 7.3. Some enzymes requiring PLP as coenzyme.

Enzymes	Reactions catalyzed
Glutamate oxaloacetate transaminase	Glutamate + oxaloacetate $\rightleftharpoons$ α -ketoglutarate + aspartate
Glutamate pyruvate transaminase	Glutamate + pyruvate $\rightleftharpoons$ α -ketoglutarate + alanine
Glutamate α -decarboxylase	Glutamate $\rightarrow$ γ -aminobutyrate + CO_2
5-Hydroxytryptophan decarboxylase	5-Hydroxytryptophan $\rightarrow$ serotonin + CO_2
AmLev synthase	Glycine + succinyl-CoA $\rightarrow$ δ -aminolevulinate + CO_2 + CoA
Kynureninase	Kynurenine $\rightarrow$ alanine + 3-hydroxyanthranilate
Serine dehydratase	Serine $\rightarrow$ pyruvate + NH_3
Cysteine desulphydrase	Cysteine $\rightarrow$ pyruvate + NH_3 + H_2S
Cystathionine synthase	Homocysteine + serine $\rightarrow$ cystathionine
Cystathionine γ -lyase	Cystathionine $\rightarrow$ cysteine + α -ketobutyrate + NH_3

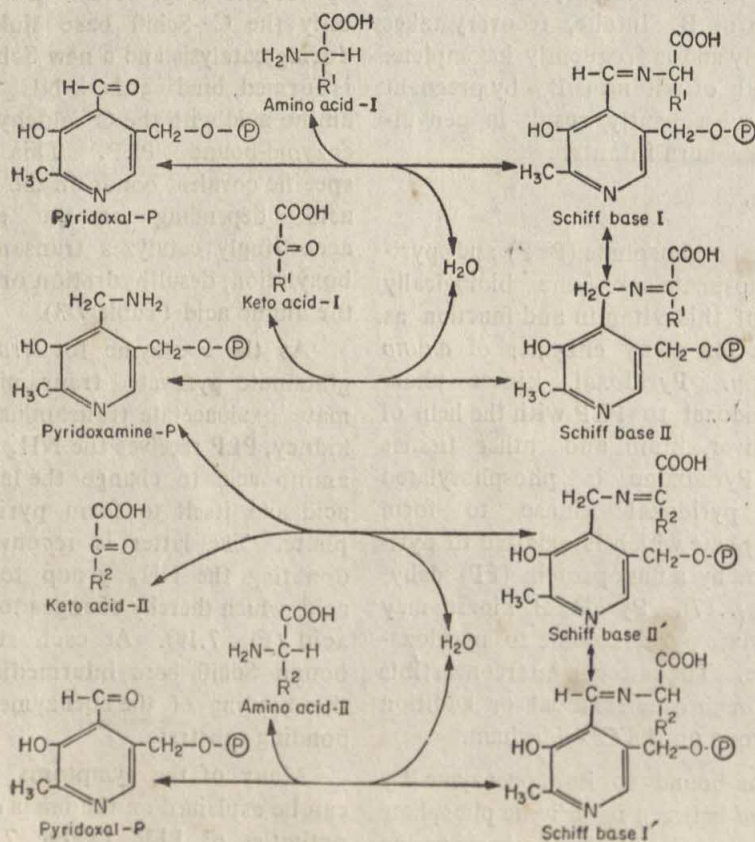


Fig. 7.19. Mode of action of PLP in transamination.

result from lowered activity of the PLP-dependent AmLev synthase, the first enzyme in porphyrin synthetic pathway. Convulsive seizures may result in pyridoxine-deficiency cases from reduced production of γ -aminobutyrate and serotonin in the nervous system due to reduced activities of glutamate α -decarboxylase and 5-hydroxytryptophan decarboxylase respectively. In B_6 -deficiency, failure of kynureninase activity prevents the normal catabolism of kynurenine, a tryptophan metabolite; kynurenine is changed instead to xanthurenate which appears in urine. Failure of kynureninase activity, due to the dearth of its coenzyme PLP, may also reduce the biosynthesis of nicotinamide from tryptophan; this may produce a secondary deficiency of nicotinamide, leading to symptoms of pellagra in B_6 -deficient patients. Urinary eliminations of homocystine and cystathionine may result from the failure of activity of the PLP-dependent enzymes, cystathionine synthase and cystathionine γ -lyase respectively, which normally catabolize homocysteine and cystathionine.

Because of the role of B_6 in amino acid metabolism, its dietary requirements increase if the food is rich in proteins.

Assay: (a) *Microbiological method*: The total amount of pyridoxine, pyridoxal and pyridoxamine in a sample may be estimated from the increase in turbidity, measured by a densitometer or photoelectric turbidimeter in a culture of *Saccharomyces carlsbergensis*, due to the growth stimulating effects of those compounds. Other microorganisms such as *Lactobacillus casei* and *Streptococcus fecalis* R are used for the estimation of the individual compounds. (b) *Biological methods*: Curative effects of vitamin B_6 compounds on dermatitis in pyridoxine-deficient rats or growth-stimulating effects of those compounds in rats are utilized for estimating the vitamin in the test material. (c) *Fluorometric method*: Different B_6 compounds are estimated by

measuring the intensities of their fluorescence at specific wavelengths.

7.6 BIOTIN

Sources: Yeast, marmite, liver, egg-yolk, kidney, dry legumes, molasses, peanuts, chocolate, tomatoes and milk. Considerable amounts are also synthesized by intestinal bacteria.

Requirements: About 200 μ g per day.

Chemistry: Biotin is hexahydro-2-oxo-1-thieno-3,4-imidazole-4-valeric acid (Fig. 7.20). It is a monobasic acid ($C_{10}H_{16}O_3N_2S$) with an n-valeric acid sidechain and fused thiophene and imidazole rings. Biotin forms colorless needle-shaped crystals (m.p. 230-232°C). It is slightly soluble in water, photostable, thermostable and acid-labile.

Absorption: Biotin is absorbed mainly from ileum. Raw egg-white of food considerably reduces the intestinal absorption of biotin. Raw egg-white contains a thermolabile basic tetrameric glycoprotein, called *avidin* (MW 70 kdal); each avidin molecule forms an indigestible, insoluble and unabsorbable complex with two molecules of biotin, leading to biotin deficiency. Cooked egg-white does not reduce biotin absorption, because heat-denatured avidin cannot bind biotin.

Deficiency: Biotin deficiency may be produced in rats by biotin-deficient diets along with sulfa drugs to kill intestinal microflora, or by feeding raw egg-white (*egg-white injury*). In rats, biotin deficiency produces poor growth, dermatitis, loss of hair, gastrointestinal lesions, loss of muscular control, incoordination and paralysis. In human volunteers,

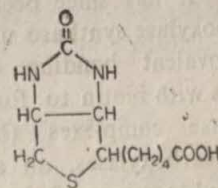


Fig. 7.20. Biotin.

TABLE 7.4. Some enzymes requiring biotin as the prosthetic group.

Enzymes	Reactions catalyzed	Metabolic roles
Pyruvate carboxylase	Pyruvate + HCO_3^- + ATP $\rightarrow$ oxaloacetate + ADP + Pi	Gluconeogenesis; anaplerosis for TCA cycle
Acetyl-CoA carboxylase	Acetyl-CoA + HCO_3^- + ATP $\rightarrow$ malonyl-CoA + ADP + Pi	Fatty acid synthesis
Propionyl-CoA carboxylase	Propionyl-CoA + HCO_3^- + ATP $\rightarrow$ D-methylmalonyl-CoA + ADP + Pi	Gluconeogenesis; propionate oxidation
β -Methylcrotonyl-CoA carboxylase	β -Methylcrotonyl-CoA + HCO_3^- + ATP $\rightarrow$ β -methylglutaconyl-CoA + ADP + Pi	Leucine metabolism

raw egg-white feeding leads to low urinary biotin, nausea, depression, easy fatigue, incoordination of movements, hyperaesthesia and dermatitis. Clinical cases of deficiency are very rare in man. In a rare *genetic deficiency of holocarboxylase synthase* which helps to utilize biotin in metabolic roles, the affected child fails to utilize biotin and consequently suffers from dermatitis, loss of hair, poor growth, incoordination of movements and high urinary lactate, β -hydroxypropionate and β -methylcrotonate.

Functions :

Biotin functions as the prosthetic group of *ATP-dependent carboxylases* such as pyruvate carboxylase, acetyl-CoA carboxylase, propionyl-CoA carboxylase and β -methylcrotonyl-CoA-carboxylase. These enzymes use HCO_3^- to carboxylate respective substrates (Table 7.4). So, biotin helps in CO_2 -fixation.

Biotin was found to remain bound with lysine residues of tissue proteins by amide bonds. *Biocytin* (ϵ -N-biotinyl-lysine) was released on hydrolyzing the peptide bonds between the biotin-bound lysine and the peptide chain. It has since been established that a holocarboxylase synthase uses ATP to catalyze the covalent bonding of different apocarboxylases with biotin to form different biotin-carboxylase complexes (holocarboxylases). In holocarboxylases, an amide bond binds the carboxyl terminus of the valeric acid sidechain of biotin with the ϵ - NH_2 at the end

of the sidechain of a lysine residue of the apocarboxylase (Fig. 7.21). A long and

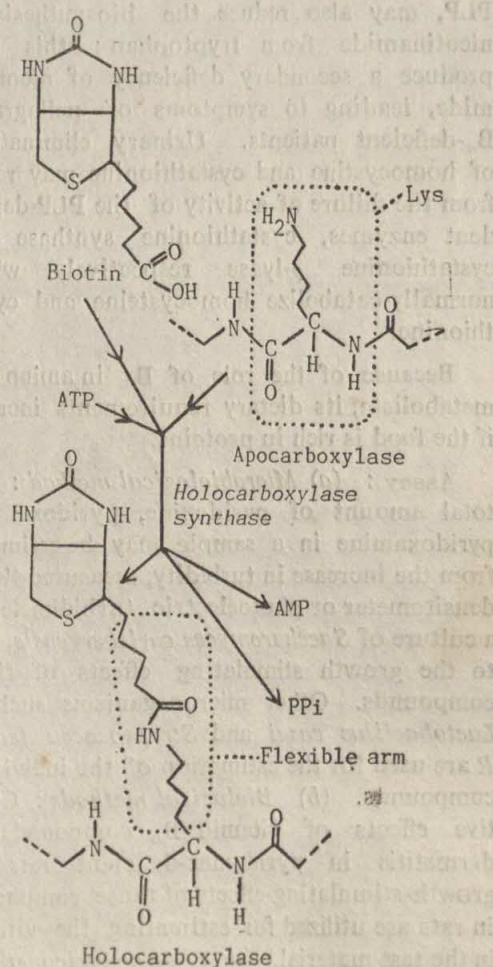


Fig. 7.21. Formation of holocarboxylase.

bacterial synthesis in the intestine or the rumen.

Daily requirements: Adult man or woman: 0.1 mg; pregnant woman: 0.3 mg; lactating woman: 0.15 mg; infants and children: 0.05-0.1 mg.

Chemistry: Folic acid or folic acid or pteroylglutamic acid (PGA) is composed of one molecule each of p-aminobenzoic acid, glutamic acid and the pigment pteridine (Fig. 7.23). It forms yellow spear-shaped crystals, slightly soluble in water. It can form metallic salts. Aqueous solutions are photolabile, absorb ultraviolet rays and emit fluorescence. Man cannot synthesize folates because of his inability to synthesize pteridine. *Folate conjugates* include pteroylheptaglutamate of yeast and plants, pteroylpentaglutamate

of liver, and pteroyltriglutamate stimulating the fermentation by *Lactobacillus casei*; these carry respectively 7, 5 and 3 glutamates serially. N^5 -Formyl-5,6,7,8-tetrahydrofolate stimulates the growth of the bacteria *Leuconostoc citrovorum* and is also called *folinic acid*, *citrovorum factor* or *leucovorin*.

Deficiency: Folate deficiency may be produced by folate deficient diets along with sulfa drugs to destroy intestinal microflora, or by administration of *folate antagonists* like aminopterin and amethopterin. Folate deficiency frequently produces megaloblastic or macrocytic anemia in pregnant women. It is characterized by large, nucleated erythrocytes with low hemoglobin (*macrocytes*) and large, highly basophilic, nucleated erythrocytes with practically no hemoglobin (*megaloblasts*). *Experimental folate deficiency* in man causes megaloblastic anemia, fall in blood hemoglobin, gastrointestinal lesions and rise in urinary formate and formiminoglutamate due to disturbances in one-carbon metabolism. Macrocytic anemia and gastrointestinal lesions of sprue as also hematological symptoms of pernicious anemia are cured on folate administration, but nervous lesions of pernicious anemia do not respond to folate treatment.

Functions:

1. Folic acid helps in the *transfer and metabolism of one-carbon compounds*. It is first reduced to H_4 folate (5,6,7,8-tetrahydrofolate) by NADPH and dihydrofolate reductase (Fig. 7.24). H_4 folate is the active derivative of folic acid. It may receive C_1 groups such as formyl, methyl, methenyl, methylene and formimino groups on its N^5 and/or N^{10} from different substrates during their catabolism. H_4 folate then acts as a carrier and donor of these C_1 groups to other substrates, as the coenzyme for many one-carbon group transferases. Aminopterin and amethopterin, the folate antagonists, produce symptoms of folate deficiency by competitive inhibition

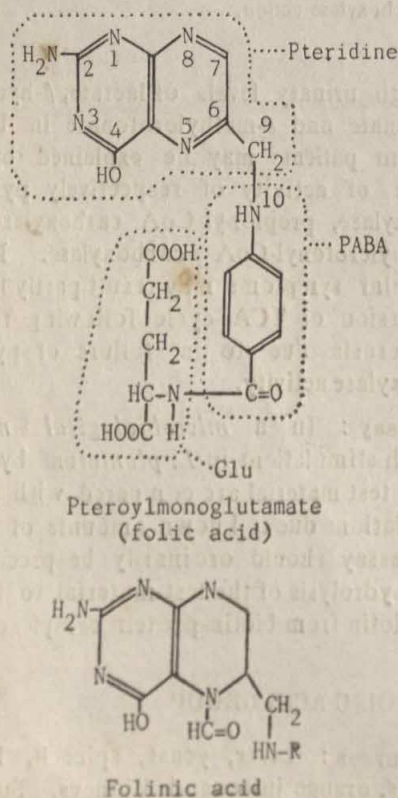


Fig. 7.23. Folic acid and folinic acid.

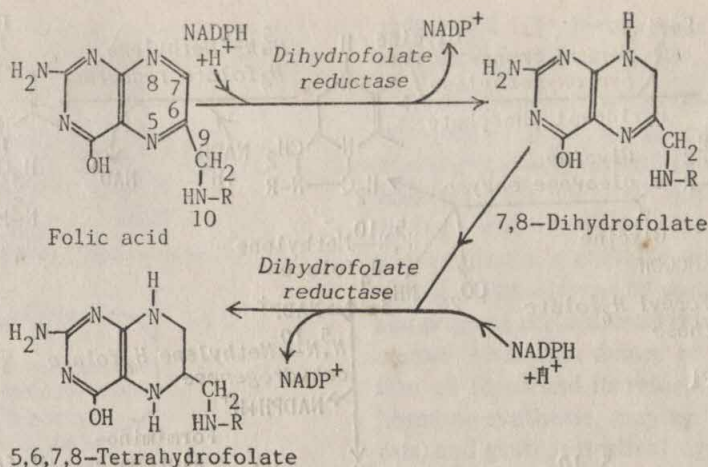


Fig. 7.24. Formation of tetrahydrofolate.

of dihydrofolate reductase which catalyzes the reduction of folate to H_4 folate.

(a) Serine transhydroxymethylase transfers the sidechain CH_2OH group of serine to H_4 folate as a methylene group (Fig. 7.25). H_4 folate and serine are thereby changed to N^5, N^{10} -methylene H_4 folate and glycine, respectively. Pyridoxal phosphate acts as a coenzyme for this reaction by forming an intermediate Schiff base with serine.

(b) Glycine cleavage enzyme uses H_4 folate, NAD^+ and pyridoxal phosphate to cleave glycine into CO_2 , NH_3 and a C_1 unit which joins H_4 folate as a methylene group to give N^5, N^{10} -methylene H_4 folate (Fig. 7.25).

(c) N^{10} -Formyl H_4 folate synthase binds formate to the N^{10} of H_4 folate with the help of ATP to produce N^{10} -formyl H_4 folate (Fig. 7.25).

(d) H_4 folate may form N^5 -formimino H_4 folate by receiving a formimino group from formiminoglutamate, a catabolite of histidine, by the action of glutamate formiminotransferase (Fig. 7.25).

Such one-carbon complexes of H_4 folate undergo interconversions in liver and other tissues. For example, N^5, N^{10} -methylene H_4 folate is oxidized to N^5, N^{10} -methenyl

H_4 folate and reduced to N^5 -methyl H_4 folate; N^5, N^{10} -methenyl H_4 folate may give rise to N^{10} -formyl H_4 folate and may also be formed from the latter.

These C_1 - H_4 folate complexes serve as donors of C_1 groups to various substrates. Some important examples are as follows:

(a) Homocysteine is methylated to methionine using N^5 -methyl H_4 folate as the ultimate methyl donor. Homocysteine methyltransferase first transfers the methyl group of N^5 -methyl H_4 folate to cobalamin, forming methylcobalamin and releasing H_4 folate (Fig. 7.26). The enzyme next transfers the methyl group from methylcobalamin to homocysteine, changing the latter to methionine. The reaction simultaneously makes H_4 folate available to act as a coenzyme for other C_1 -transfer reactions including those for purine and deoxythymidylate synthesis.

(b) C^2 and C^8 of the purine ring are derived from N^{10} -formyl H_4 folate and N^5, N^{10} -methenyl H_4 folate respectively, with the help of transformylases.

(c) N^5, N^{10} -Methylene H_4 folate donates its methylene group to deoxyuridylic acid (dUMP) under the action of thymidylate synthetase (Fig. 7.27). The methylene group is

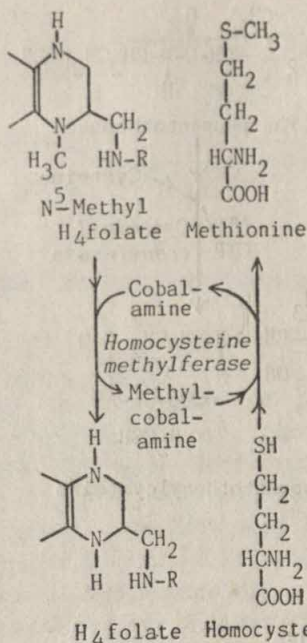


Fig. 7.26. Folate and cobalamin in the methylation of homocysteine.

to doses of the test material are estimated either by measuring the turbidity of the medium by a photoelectric turbidimeter or by titrating the lactic acid produced; the results are then compared with analogous effects of graded doses of standard folate preparations. In another microbiological method, growth stimulations of *Streptococcus fecalis* due to doses of the test material are compared with similar effects of standard folate preparations, with the help of a photoelectric turbidimeter.

7.8 PANTOTHENIC ACID

Sources: Yeast, liver, kidney, egg-yolk, legumes and nuts. Sufficient amounts may also be synthesized by intestinal bacteria.

Requirements: Probably 4-10 mg per day.

Chemistry: Pantothenic acid or α, γ -dihydroxy- β, β -dimethylbutyryl- β' -alanide (Fig. 7.28) is a water-soluble, thermolabile, pale

yellow oil. It forms salts with metals and monoesters with alcohols.

Deficiency: Pantothenate deficiency in rats causes poor growth, dermatitis, loss of hair particularly around eyes, reddish color of whiskers and of the skin under the eye, aplastic bone marrow, leucopenia, anemia, necrosis and low cholesterol content of adrenal cortex, dehydration and disturbances in water and mineral metabolisms due to poor corticosteroid secretion, failure of uterine implantation of fetus and its resorption due to low sex hormone synthesis, graying of hair (in black rats) and gastrointestinal symptoms. Experimental deficiency of pantothenate in human volunteers produces gastrointestinal symptoms, easy fatigue, changed sleep pattern, staggering gait and mental symptoms. Administration

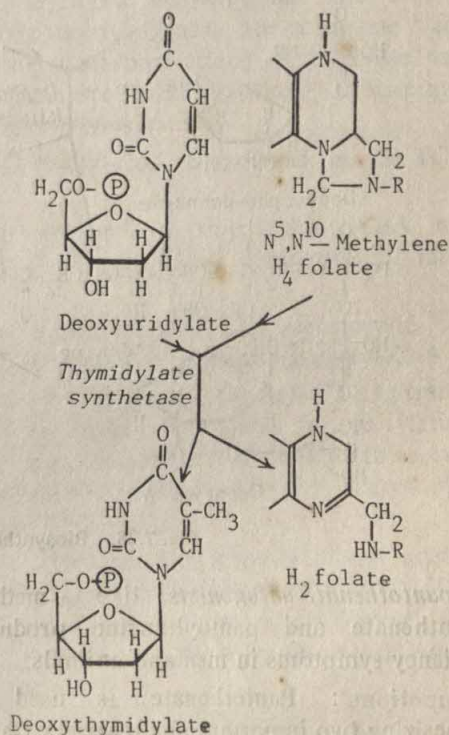


Fig. 7.27. Methylation of deoxyuridylylate (dUMP) to deoxythymidylylate.

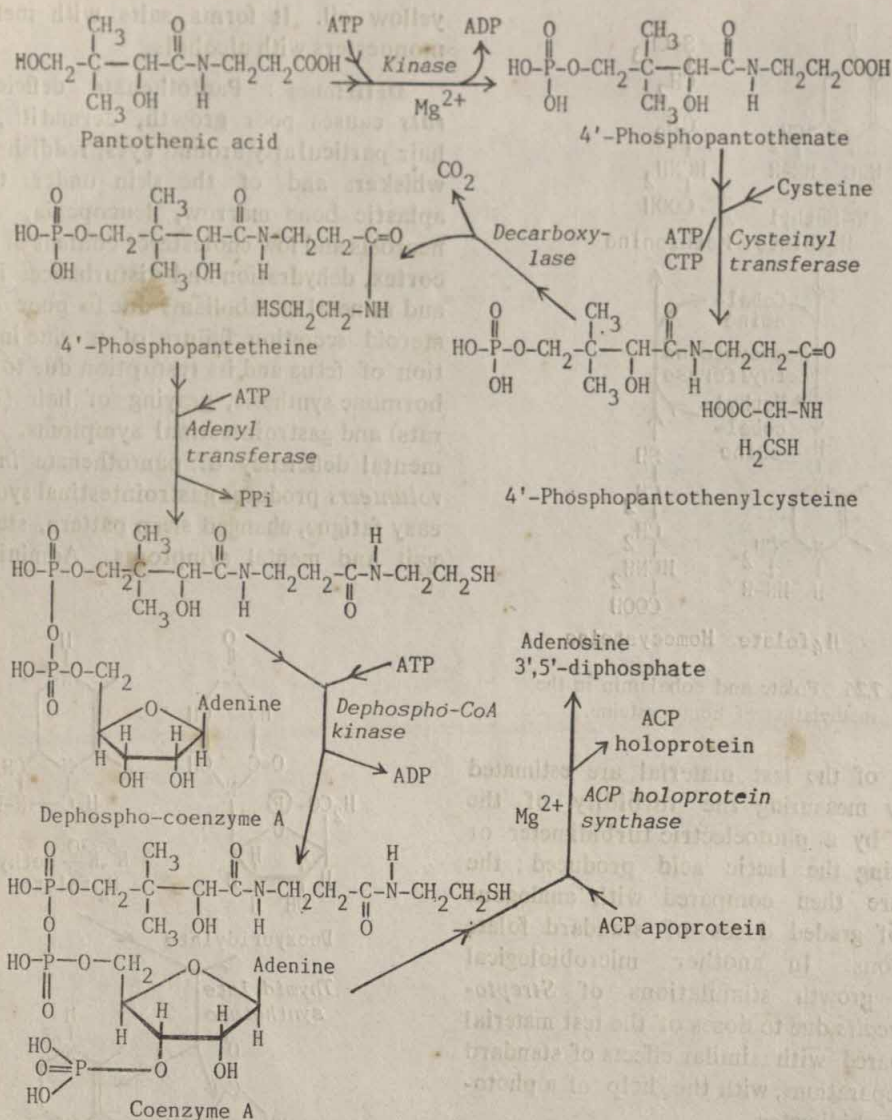


Fig. 7.28. Biosynthesis of coenzyme A and ACP.

of pantothenate antagonists like ω -methyl-pantothenate and pantooyltaurine produces deficiency symptoms in men and animals.

Functions: Pantothenate is used in synthesizing two important acyl-carriers (fatty acid carriers), viz., coenzyme A and acyl-carrier protein (ACP). Both molecules carry

4'-phosphopantetheine formed from pantothenate (Fig. 7.28). In both, the terminal SH group of 4'-phosphopantetheine may bind fatty acyl groups by thioester linkages and carry them for transfer or metabolism.

1. **Coenzyme A:** This functions widely as an acyl-carrier. Thus, it is a coenzyme

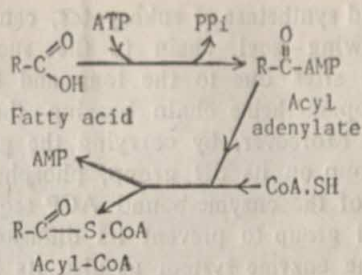


Fig. 7.29. Action of thiokinase.

for the synthesis, elongation and oxidation of fatty acids, oxidative decarboxylations, acetylations, and synthesis of porphyrins, sterols, triglycerides and phospholipids. Fatty acid thiokinase first transfers an adenylate (AMP) group from ATP to the carboxyl group of a fatty acid, forming an acyl adenylate (fatty acid-AMP) and releasing an inorganic pyrophosphate; then, the enzyme produces a high-energy thioester bond between the SH group of coenzyme A and the carboxyl group of the fatty acid, releasing the AMP and producing an acyl-CoA (Fig. 7.29). The over-all reaction is driven irreversibly by the immediate hydrolysis of PPi to two Pi molecules. To a minor extent, thiophorases form some acyl-CoA by replacing the acyl group of a pre-existing acyl-CoA by a new fatty acid (Fig. 7.30). Thiokinases and thiophorases produce acyl-CoA like palmitoyl-CoA and acetyl-CoA. Coenzyme A is also used by thiolase (β -ketothiolase) for the *thiolysis* of β -keto acids to produce acetyl-CoA molecules

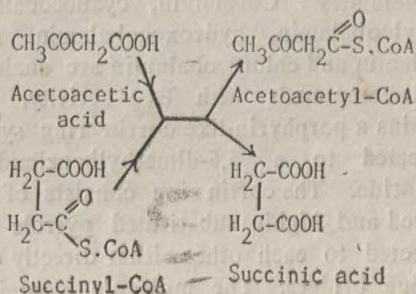


Fig. 7.30. Action of a thiophorase.

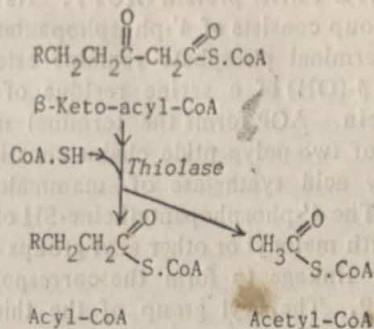


Fig. 7.31. Action of thiolase.

(Fig. 7.31). Some examples of the use and metabolism of acyl-CoA complexes are cited below to illustrate the metabolic roles of coenzyme A.

(a) Acyl-CoA is used for β -oxidation of its acyl group, yielding acetyl-CoA molecules.

(b) Pyruvate dehydrogenase and α -ketoglutarate dehydrogenase use coenzyme A for oxidative decarboxylations of pyruvate and α -ketoglutarate into acetyl-CoA and succinyl-CoA respectively.

(c) Acetyl-CoA is oxidized in the TCA cycle.

(d) Acetyl-CoA and malonyl-CoA are used in the synthesis and elongation of fatty acids.

(e) Ketone bodies like acetoacetate are formed and catabolized through acetyl-CoA.

(f) Acetyl group of acetyl-CoA is transferred to various substrates for acetylating them; e.g., acetylation of choline to acetylcholine and of sulfanilamide to N-acetylsulfanilamide (Fig. 7.32).

(g) HMG-CoA, synthesized from acetyl-CoA, is used in cholesterol synthesis. This explains the low adrenal cholesterol and the cortical insufficiency in pantothenate deficiency.

(h) Succinyl-CoA is used in heme synthesis. This explains the anemia in pantothenate deficiency.

2. **Acyl-carrier protein (ACP):** Its prosthetic group consists of 4'-phosphopantetheine whose terminal phosphate remains esterified with the β -(OH) of a serine residue of ACP apoprotein. ACP forms the terminal moiety of each of two polypeptide chains constituting the fatty acid synthetase of mammals and birds. The 4'-phosphopantetheine-SH of ACP binds with malonyl or other acyl groups by a thioester linkage to form the corresponding acyl-ACP. The acyl group of the thioester complex is then progressively built up into longer acyl groups by fatty acid synthetase. The 4'-phosphopantetheine chain of ACP measures about 2 nm in length, when fully extended. ACP, bound to the multienzyme

fatty acid synthetase of eukaryotes, can bring the growing acyl chain to the successive enzymic sites due to the long and flexible phosphopantetheine chain bearing the acyl group. Moreover, by carrying the growing acyl group on its SH group, phosphopantetheine of the enzyme-bound ACP sequesters the acyl group to prevent its diffusion away from the enzyme system as also its loss in other competing reactions.

Assay: (a) *Microbiological method:* The growth stimulating effects of the test material on *Lactobacillus plantarum* are estimated either by titration of the lactic acid produced or by measurement of the increase in the turbidity due to a rise in the bacterial population in the culture; these are compared with similar effects of known amounts of the vitamin on the bacteria. (b) *Colorimetric method:* Pantothenyl alcohol may be estimated from the intensity of color produced by its reaction with 1,2-naphthoquinone 4-sulfonate.

7.9 VITAMIN B₁₂ OR COBALAMIN

The vitamin is also known as the anti-pernicious anemia factor, *Lactobacillus lactis* Dorner factor and Castle's extrinsic factor.

Sources: Animal proteins like meat, fish, kidney, liver. The vitamin is synthesized by bacteria in human colon and bovine rumen.

Daily requirements: Adult man or woman: 1 μ g; pregnant or lactating woman: 1.5 μ g; infants and children: 0.2-1 μ g.

Chemistry: Cobalamin, cyanocobalamin, methylcobalamin, hydroxocobalamin, nitrocobalamin and chlorocobalamin are analogous cobalt-compounds with B₁₂ activity. Each contains a porphyrin-like corrin ring system, connected to a 5,6-dimethylbenzimidazole nucleotide. The corrin ring consists of four reduced and highly substituted pyrrole rings connected to each other either directly or by methene bridges. The molecule contains a cobalt ion bonded to the four pyrrole nitrogens

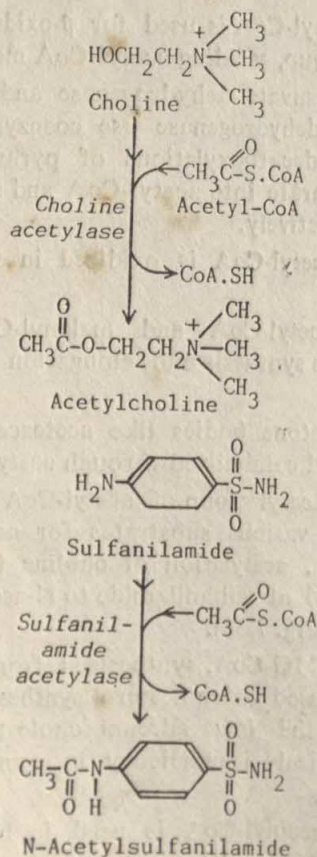


Fig. 7.32. Some acetylations using coenzyme A.

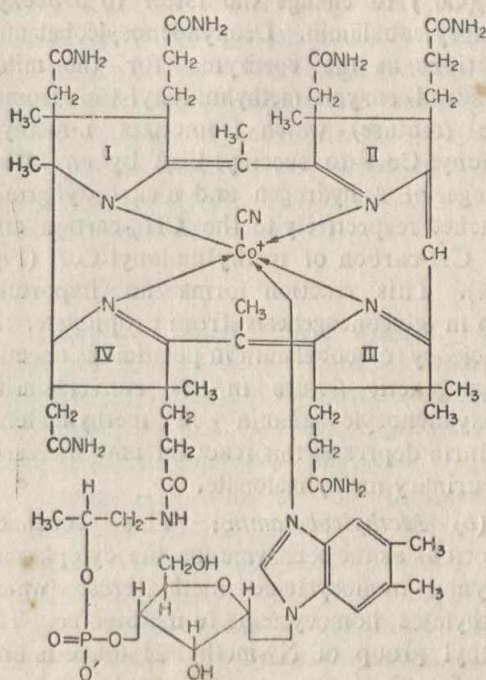


Fig. 7.33. Cyanocobalamin.

and also to a nitrogen of the benzimidazole ring. The cobalt is also linked to OH^- , CN^- , CH_3 or 5'-deoxyadenosyl group in hydroxocobalamin, cyanocobalamin, methylcobalamin and deoxyadenosylcobalamin respectively.

Cyanocobalamin (Fig. 7.33) forms tasteless, odourless, needle-shaped, red crystals, soluble in water and fairly thermostable. Its cyano group is an isolation artifact.

Absorption : B_{12} is actively absorbed from ileum by a specific transport system. B_{12} absorption is largely helped by the gastric HCl and Castle's intrinsic factor (MW 50000) which is a thermolabile dimeric mucoprotein secreted by gastric parietal cells. At an acidic gastric pH, the intrinsic factor binds with B_{12} . The B_{12} -intrinsic factor complex passes without inactivation to the ileum and gets bound to a specific receptor in the microvillus membrane of intestinal epithelium in presence of divalent cations and at a near-neutral pH. B_{12} is subsequently released from

the B_{12} -intrinsic factor complex by a releasing factor of duodenal juice in presence of Ca^{2+} and is actively transported across the membrane into the brush-bordered cells of ileum. A failure of B_{12} absorption produces pernicious anemia in man.

Transport : Vitamin B_{12} is transported in the plasma in combination with globulins, called transcobalamins or transcorrins I and II, and stored in combination with transcorrin I in the liver.

Deficiency :

1. **Dietary deficiency :** This occurs rarely in strict vegetarians and is characterized by neurological symptoms, paraesthesia and glossitis.

2. **Pernicious anemia :** This is caused by a failure of intestinal absorption of cobalamins. It may result from any of the following factors, viz., a congenital or postnatal failure of intrinsic factor secretion, a congenital lack of specific B_{12} receptors in the intestinal membrane, an inhibition of parietal cells or intrinsic factor by immunoglobulins produced by autoimmunization, or gastric atrophy and achlorhydria. Pernicious anemia is characterized by neurological lesions, megaloblastic anemia, fall in serum and urinary levels of B_{12} , and rise in urinary methylmalonate. Neurological lesions include a degeneration of the spinal cord and neuronal dysfunction. Blood changes consist of reductions in the RBC count and hemoglobin content, but the plasma iron level and the mean corpuscular hemoglobin may rise. Blood as well as red bone marrow contains numerous abnormal and immature erythrocytes. They include normoblasts, erythroblasts, abnormally large erythrocytes called macrocytes, and large basophilic cells called megaloblasts. Megaloblasts contain large reticulated nuclei, almost no hemoglobin and abnormally high RNA : DNA ratio. The red bone marrow becomes hyperplastic. Intramuscular injections of B_{12} cure

both neurological and hematological symptoms.

3. **Methylmalonic aciduria**: Some cases of methylmalonic aciduria result from a genetic failure to convert cobalamin to 5'-deoxyadenosylcobalamin, a coenzyme. The consequent deficiency of cobalamin activity leads to high urinary methylmalonic acid, acidosis and a fall of blood pH to 7.0. Parenteral administration of large doses of cobalamin ameliorates the symptoms, provided there is no genetic deficiency of methylmalonyl-CoA isomerase, the apoenzyme for 5'-deoxyadenosylcobalamin.

Functions :

Cobalamin is changed in tissue cells to two principal *cobamide coenzymes*, viz., methylcobalamin in the cytosol and 5'-deoxyadenosylcobalamin in the mitochondria.

(a) **5'-Deoxyadenosylcobalamin**: Hydroxocobalamin or $B_{12a}(Co^{3+})$ is reduced stepwise by two flavoprotein reductases and NADPH to give $B_{12s}(Co^{+})$. An adenosyltransferase then transfers 5'-deoxyadenosyl group from ATP to

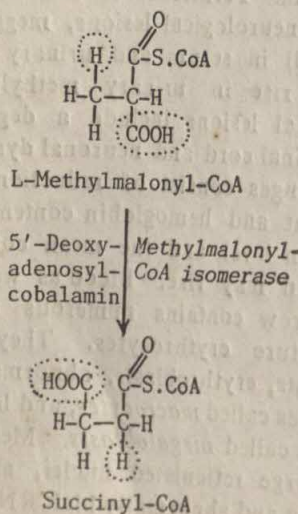


Fig. 7.34. Action of methylmalonyl-CoA isomerase.

$B_{12s}(Co^{+})$ to change the latter to 5'-deoxyadenosylcobalamin. Deoxyadenosylcobalamin functions as the coenzyme for the mitochondrial enzyme methylmalonyl-CoA isomerase (mutase) which isomerizes L-methylmalonyl-CoA to succinyl-CoA by an interchange of a hydrogen and a carboxyl group attached respectively to the CH_3 -carbon and the CH-carbon of methylmalonyl-CoA (Fig. 7.34). This reaction forms an important step in gluconeogenesis from propionate. A deficiency of cobalamin in pernicious anemia, or a genetic failure in its conversion to deoxyadenosylcobalamin in methylmalonic aciduria depresses this reaction and increases the urinary methylmalonate.

(b) **Methylcobalamin**: This cobamide functions as the coenzyme for the cytoplasmic enzyme homocysteine methyltransferase which methylates homocysteine to methionine. The methyl group of N^5 -methyl H_4 folate is first transferred to the enzyme-bound cobalamin, changing the latter to methylcobalamin. The latter then donates its methyl group to homocysteine (Fig. 7.26). In pernicious anemia, a failure of this reaction due to B_{12} deficiency causes a deficiency of methionine which is needed for transmethylation of phosphatidylethanolamines to phosphatidylcholines in forming membranes and myelin. This may be a factor in neurological lesions seen in pernicious anemia. Moreover, a failure of the homocysteine methyltransferase action owing to B_{12} deficiency reduces the reconversion of N^5 -methyl H_4 folate to H_4 folate. This results in the nonavailability of H_4 folate for coenzyme functions in purine and deoxythymidylate syntheses (vide 7.7); this secondarily reduces DNA synthesis and depresses red cell maturation, leading to megaloblastic anemia.

Assay: In the *microbiological method*, the growth stimulations in cultures of a mutant strain of *Escherichium coli*, on addition of the test material and of standard cobalamin,

preparations, are compared by measuring the diameters of the growth zones in the agar plate. In another microbiological method, the growth promoting effects of the test material and a standard cyanocobalamin solution on *L. leichmani* cultures are estimated by the electrophotometric determination of their transmittances and compared.

7.10 INOSITOL

Sources: Yeast, liver, kidney, cereal grains, nuts, soyabean. Inositol hexaphosphate or phytic acid of wheat and oats reduces the absorption of calcium, magnesium and iron by forming insoluble phytates.

Chemistry: Inositol or hexahydroxycyclohexane ($C_6H_{12}O_6$) is a polyhydroxy alcohol. Its optically inactive isomer, meso-inositol, acts as a vitamin (Fig. 7.35). Meso-inositol forms white water-soluble crystals (m.p. 225°C). Its hydroxyl groups may be esterified with acids like phosphoric acid.

Deficiency: Inositol deficiency in mice causes poor body-growth, loss of hair particularly around the eye (spectacled-eye appearance), fatty infiltration of the liver and failure of lactation. The symptoms are cured by administration of inositol (*mouse anti-alopecia factor*).

Functions:

1. It is used in the synthesis of *phosphatidylinositols*, important membrane lipids.
2. Some inositol polyphosphates such as inositol 1,3,4,5-tetraphosphate may act as *second messengers* mediating in the cellular effects of specific informational molecules from the extracellular medium. Irvine, Moor,

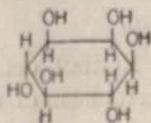


Fig. 7.35. Meso-inositol.

Michell and others postulate that such informational molecules may bind with specific molecular receptors in the plasma membrane and may thereby induce the synthesis of inositol 1,3,4,5- P_4 . The latter may then enhance Ca^{2+} inflow into the cell from the extracellular fluid, leading to various cellular changes and activities. This idea has been mooted from their work on sea urchin egg cells.

3. Inositol is a *lipotropic factor*. It prevents accumulation of excess fat and cholesterol in the liver by promoting their mobilization from the liver, probably through the formation of phosphatidylinositols.

Assay: In the *microbiological method*, the growth rate of *Saccharomyces cerevisiae* in response to graded amounts of the test material is compared to the rate of growth in presence of known amounts of the vitamin. The stimulating effect of inositol on a mutant strain of *Neurospora crassa* is the basis for another microbiological method.

7.11 VITAMIN A OR RETINOL

Sources: Vitamin A is present in fish liver oils such as cod, mackerel and halibut liver oils, shark liver oil, liver, egg-yolk, milk, butter, clarified butter (ghee), cheese, lard and flesh of oily fishes like hilsa and herring. Hydrogenated vegetable oils are 'fortified' with 700 IU of vitamin A per ounce. Vitamin A is also formed in the body from carotenes (*provitamins A*). These are yellow pigments abundant in yellow crops, fruits and vegetables such as maize and carrot, and dark-green leaves like ipomea leaves, radish tops and spinach.

Daily requirements: Adult man, adult woman or pregnant woman: 750 μg of retinol or 3 mg of β -carotene; lactating woman: 1.15 mg of retinol or 4.6 mg of β -carotene; infants or children: 400-750 μg of retinol. 1 IU (international unit) is equivalent to 0.3 μg of retinol or 0.6 μg of β -carotene.

Chemistry: Vitamins A are polyisoprenoid compounds, each with a β -ionone ring and several double bonds. Vitamin A₁ or *retinol*₁ occurs in tissues of mammals and marine fishes. It forms pale-yellow prism-shaped crystals (m.p. 63-64°C), insoluble in water and soluble in ether, chloroform and acetone (Fig. 7.36). Vitamin A₂ or *retinol*₂ or 3-dehydroretinol₁ occurs in livers of freshwater Indian fishes. It has an additional double bond and only 40% biological activity compared to retinol₁. Retinol₁ and retinol₂ absorb ultraviolet light with absorption maxima at 328 and 351 nm respectively. Both are thermostable, but inactivated by ultraviolet rays and aerobic oxidation. Both form esters with fatty acids through the terminal alcohol

group. All-*trans*-retinols are retinol isomers with all their double bonds in *trans* configuration.

Carotenes are hydrocarbons, forming yellow to dark-red fat-soluble crystals; α -, β - and γ -carotenes respectively have mps of 187°, 184° and 176°C, and carry one, two and one β -ionone rings.

Absorption: Vitamin A esters are hydrolyzed by pancreatic cholesterol esterase into fatty acids and free retinol. Free retinol is absorbed from the proximal small intestine without the help of bile salts. But carotenes are incorporated by bile salts into water-soluble micelles and absorbed in such forms from the intestine.

Transport: The absorbed vitamin A is esterified mainly with palmitic acid in the intestinal mucosa and transported in chylomicron droplets of lymph to the liver for storage as retinoyl palmitate.

Synthesis from carotenes: Carotenes are changed to retinol mainly in the intestinal mucosa. In presence of bile salts, β -carotene 15,15'-dioxygenase oxidatively cleaves the central C=C double bond of β -carotene to change it to two all-*trans*-retinal molecules having all the double bonds in the *trans* configuration. All-*trans*-retinal is mainly reduced to retinol by retinal reductase (retinene reductase) and NADPH, but is also oxidized to a minor extent to retinoic acid (Fig. 7.37). α - or γ -carotene carries only one β -ionone ring and consequently gives a single retinol molecule by similar reactions. Carnivores cannot utilize carotenes.

Fate: Retinol is excreted as retinoic acid in the bile and as retinoyl uronides and epoxides in the urine. Retinol is secreted in milk and colostrum.

Deficiency: Retinol deficiency produces a keratinizing metaplasia of epithelia in man. The normal living epithelial tissues are replaced by flattened, keratinized, heaped and

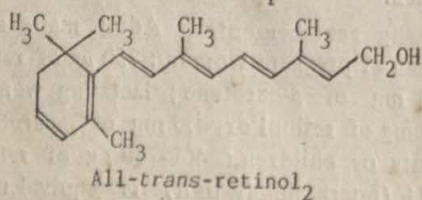
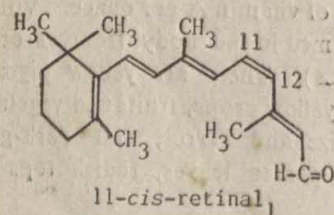
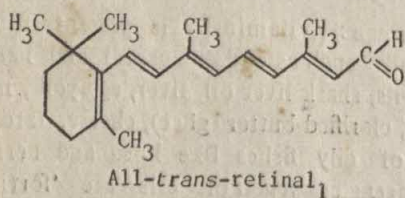
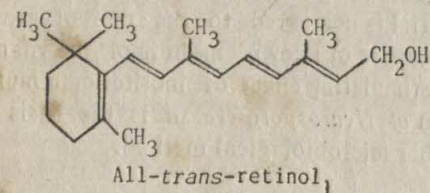


Fig. 7.36. Retinols and retinals.

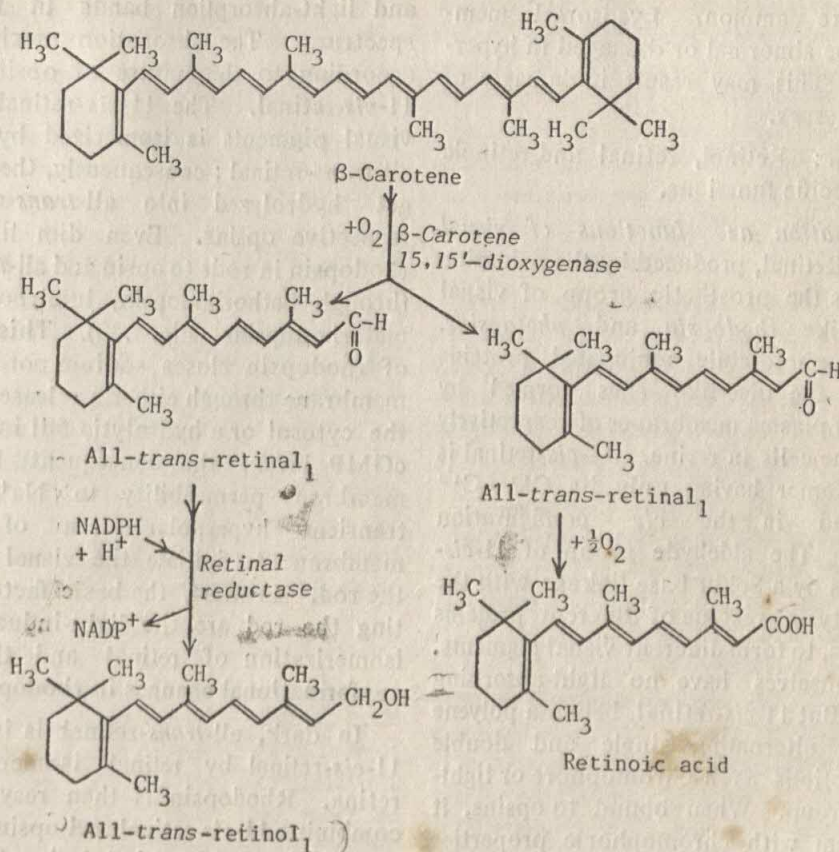


Fig. 7.37. Biosynthesis of retinoids.

stratified epithelial cells. Sweat and sebaceous glands atrophy and get keratinized. Corn-like keratinized masses appear in the skin. Keratinization of lachrymal glands, suppression of lachrymation and keratinization of ophthalmic epithelium (*xerophthalmia*) lead to conjunctivitis, thickening, drying and softening of cornea, ulceration of corneal epithelium and finally, blindness. Vision in dim light becomes defective (*nyctalopia* or night-blindness). Secretory epithelium of renal tubules gets keratinized. This leads to secondary infections and renal calculi. Keratinization of gonads causes reproductive failures. Poor or abnormal bone growth in cranium and vertebrae retards body growth

and growth of the nervous system, and damages the nervous system by bone pressure.

A rare type of retinol deficiency with low plasma retinol, high plasma β -carotene and night-blindness, may result from a *genetic deficiency of carotene dioxygenase*.

Hypervitaminosis A : This results from massive administration of retinols, but not of carotenes. In chronic cases, nausea, irritability, headache, abnormal calcifications, painful swellings over bones, limitations of limb movements, loss of hair, dermatitis, dizziness and blurring of vision may be observed. In acute cases, nausea, diplopia, nasal bleedings, drowsiness, headache, peeling of skin and

dermatitis are common. Lysosomal membranes may be abnormal or damaged in hypervitaminosis. This may result in damages of connective tissues.

Functions : Retinol, retinal and retinoic acid have specific functions.

1. **Formation and functions of visual pigments :** Retinal, produced by the oxidation of retinol, is the prosthetic group of visual pigments like *rhodopsin* and *photopsins*. These are photolabile conjugated proteins attached to the disc-like sacs formed by infoldings of plasma membranes of respectively rod and cone cells in retina. 11-*cis*-retinal is a retinal isomer having only its $C^{11}=C^{12}$ double bond in the *cis* configuration (Fig. 7.36). The aldehyde group of 11-*cis*-retinal binds by a Schiff base linkage with the ϵ -NH₂ of a lysine residue of different proteins called opsins, to form different visual pigments. Opsins themselves have no light-absorbing property. But 11-*cis*-retinal, being a polyene with many alternating single and double bonds, functions as a chromophore or light-absorbing group. When bound to opsins, it endows them with chromophoric properties

and light-absorption bands in the visible spectrum. The absorption maximum varies according to the nature of opsin bound to 11-*cis*-retinal. The 11-*cis*-retinal group of visual pigments is isomerized by light into all-*trans*-retinal; consequently, these pigments get hydrolyzed into all-*trans*-retinal and respective opsins. Even dim light breaks rhodopsin in rods to opsin and all-*trans*-retinal through bathorhodopsin, lumirhodopsin and metarhodopsins (Fig. 7.38). This photolysis of rhodopsin closes sodium pores in the rod membrane through either a release of Ca^{2+} in the cytosol or a hydrolytic fall in the cytosol cGMP level; the consequent lowering in membrane permeability to Na^{+} causes a transient hyperpolarization of the rod membrane to initiate the visual signal from the rod. In short, the basic factors stimulating the rod are the light-induced *cis-trans* isomerization of retinal and the resulting conformational changes in rhodopsin.

In dark, all-*trans*-retinal is isomerized to 11-*cis*-retinal by retinal isomerase in the retina. Rhodopsin is then resynthesized by combining 11-*cis*-retinal and opsin. But some retinal liberated by photolysis of rhodopsin, is not available for rhodopsin resynthesis and must be replaced by synthesizing fresh 11-*cis*-retinal from retinol. For this, all-*trans*-retinol is collected from blood and oxidized by retinol dehydrogenase and $NADP^{+}$ to all-*trans*-retinal. The latter is then isomerized to 11-*cis*-retinal by retinal isomerase and incorporated into rhodopsin. So, retinol deficiency depresses the resynthesis of rhodopsin and interferes with the function of rods resulting in night-blindness.

2. **Maintenance of living epithelia :** Retinoic acid, formed by the oxidation of retinol and retinal, seems to stimulate the differentiation, growth and maintenance of normal living secretory epithelia and to prevent their atrophy and keratinization. This may be mediated partly by a stimulation

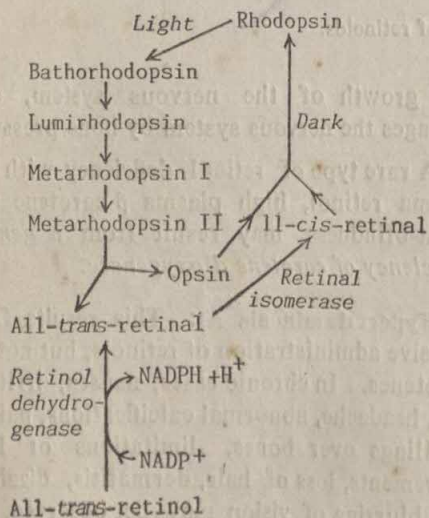


Fig. 7.38. Photolysis and resynthesis of rhodopsin in retina.

of glycoprotein synthesis and partly by a rise in density of receptors for growth-stimulating factors, on the epithelial cell membrane.

3. *Glycoprotein synthesis*: Retinoic acid stimulates glycoprotein synthesis, probably by forming a metabolite like retinoyl phosphate. The latter may act as a carrier of oligosaccharides in glycoprotein synthesis. The retinoic acid metabolite may also bind with and carry phosphohexoses like mannose and galactose phosphates. It may also carry oligosaccharides across membrane lipids. Hypovitaminosis A reduces mucoprotein formation, depresses the incorporation of mannose in liver glycoproteins, causes the accumulation of small lipo-oligosaccharide complexes in the liver, and impairs the development and maintenance of the ground substance in collagenous tissues. All these can be rectified by retinoic acid.

4. *Maintenance of reproductive function*: Retinol itself appears to promote normal reproduction by acting on the nuclear gene through a *mobile receptor model* as in case of steroid hormones. It binds with a cytoplasmic retinol-binding protein which then goes to bind with some nuclear proteins in specific genes. This changes gene transcription to influence the reproductive organs.

Assay: In the *spectrophotometric method*, the absorbancies of ultraviolet rays (at about 325 nm) by the test solution and by the pure solvent, are measured with a spectrophotometer. In the *colorimetric method*, the intensity of the blue color produced by the addition of antimony trichloride to the test sample is measured in a photoelectric colorimeter. In a *biological method of assay*, the growth responses of vitamin A-deficient young rats, on administration of the test material and known amounts of vitamin A, are compared.

7.12 VITAMIN D

Sources: Marine fish liver oils like cod liver oil, oily flesh of fishes such as herring,

sardine and salmon, liver and viscera of many fishes, shark liver oil, shrimps and egg-yolk contain vitamin D. Cow's milk is often fortified with this vitamin. Hydrogenated vegetable fats are also fortified with 70 IU (international unit) of vitamin D per ounce. Sufficient amounts may also be synthesized in the epidermis of skin by the effect of ultraviolet rays of sunlight.

Daily requirements: Adult man or woman: 100 IU or 2.5 μg of vitamin D_3 —easily supplied by cutaneous biosynthesis in sunlit tropical countries; infants or children, pregnant or lactating woman: 5 μg or 200 IU of D_3 —to be supplied in the daily diet.

Chemistry: Vitamin D_3 or *cholecalciferol* occurs in fish livers and is also produced in human skin by ultraviolet irradiation of 7-dehydrocholesterol (Fig. 7.39). Vitamin D_2 or *ergocalciferol* is obtained by ultraviolet irradiation of ergosterol of yeast or ergot. Vitamins D are white, crystalline steroid-derivatives, soluble in fat-solvents like chloroform and ether, thermostable and resistant to oxidation. Vitamin D_3 or cholecalciferol melts at 83°C, can be hydrogenated and exhibits absorption maxima at about 265 nm. Ergosterol and 7-dehydrocholesterol are called *provitamins D*.

Absorption: Bile salts help in the absorption of vitamin D from duodenum and jejunum by incorporating the vitamin in water-soluble micelles. Failure in the entry of bile into the duodenum reduces vitamin D absorption. After absorption, vitamin D is carried in chylomicron droplets of the lymph, but in combination with serum globulin in blood plasma.

Synthesis: In man, provitamin D_3 or 7-dehydrocholesterol is partly obtained from food and partly synthesized in skin or intestinal mucosa. It is photolyzed by ultraviolet rays (λ 265-300 nm) to precholecalciferol in the epidermis—the ring B is opened by the action of ultraviolet light. Precholecalciferol

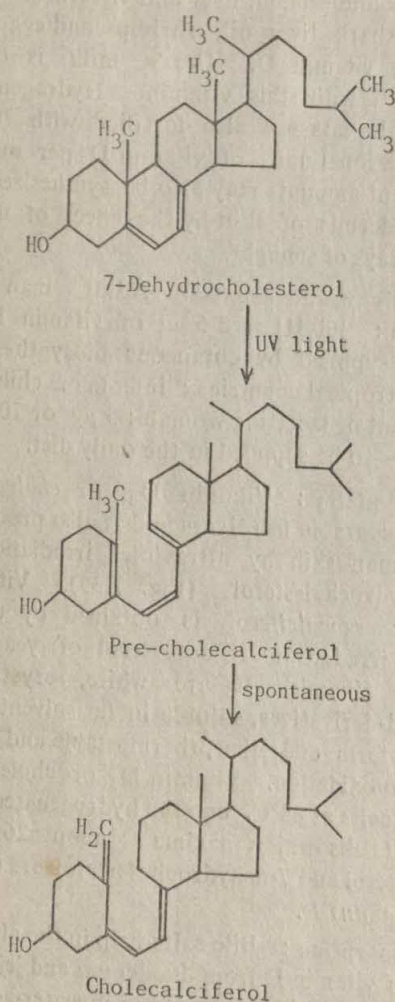


Fig. 7.39. Biosynthesis of cholecalciferol.

spontaneously isomerizes to cholecalciferol. Ergosterol is similarly photolyzed to vitamin D_2 by UV rays. But overexposure to the latter may convert the vitamin to inactive or toxic products like toxisterol and suprasterols.

Active derivatives: Cholecalciferol is hydroxylated in the liver microsomes by vitamin D_3 25-hydroxylase to form 25-hydroxycholecalciferol (25-HCC) and stored (Fig. 7.40); corticosteroids as also a feed-back of 25-HCC may depress this hydroxylation. From the liver, 25-HCC is carried by an

α -globulin of blood to the renal tubules, bone cells and placenta. Mitochondrial 25-HCC 1 α -hydroxylase converts it to 1 α ,25-dihydroxycholecalciferol (1,25-DHCC or 1,25 $[OH]_2D_3$) in presence of NADPH, Mg^{2+} and molecular O_2 . 25-HCC and low plasma phosphate induce the synthesis of 25-HCC 1 α -hydroxylase. 1,25 $[OH]_2D_3$ causes a feed-back inhibition of the enzyme. Parathyroid hormone, secreted in consequence of a fall in serum Ca^{2+} , increases the 1 α -hydroxylase activity to enhance 1,25 $[OH]_2D_3$ synthesis. 1,25 $[OH]_2D_3$ is the principal active derivative of cholecalciferol.

But 24,25-DHCC may be formed from 25-HCC by the 25-HCC 24-hydroxylase in the mitochondria of renal tubules, intestinal cells, bone cells, chondrocytes and placenta whenever 1,25-DHCC production falls. Parathyroid hormone and a low serum Ca^{2+} reduces the activity of 25-HCC 24-hydroxylase in renal mitochondria to depress 24,25-DHCC formation. A rise in calcitonin, a fall in parathormone or a rise in serum Ca^{2+} enhances the conversion of 25-HCC to 24,25-DHCC.

Deficiency: Vitamin D deficiency produces rickets in children and osteomalacia in adults. In tropical countries with plentiful sunlight, these diseases occur only in such sections of the population as do not receive sufficient exposure to the sun. Children employed in cottage industries, regular night-shift workers, women (particularly pregnant women) observing 'purdah' or residing in dark urban tenements, and miners may suffer from vitamin D deficiency due to the failure of cutaneous synthesis of the vitamin.

In *rickets*, poor intestinal absorption of calcium and phosphate, rise in urinary phosphate, negative calcium balance, loss of bone calcium, softness and deformities of bones (like bow-legs and pigeon-chest) and delay in the closure of fontanelle are observed. The cartilage cells at the ends of long bones continue to grow and proliferate. Formation

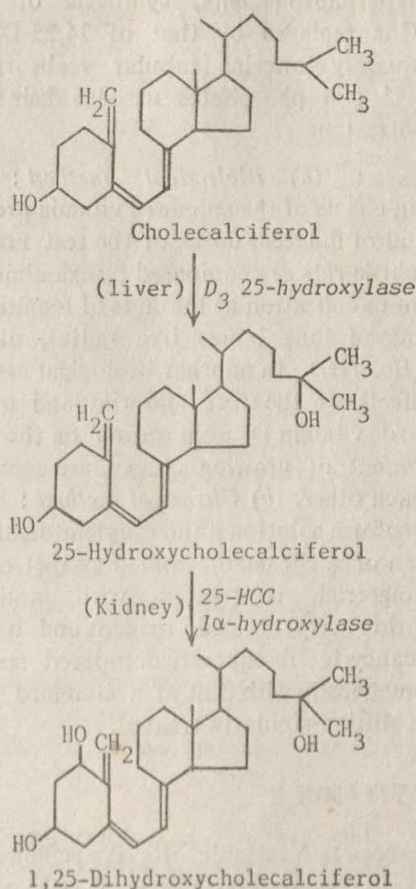


Fig. 7.40. Biosynthesis of active D₃ derivatives.

of new bone matrix also continues. But ossification of the cartilage is delayed. The enlarged cartilage tissue at the ends of long bones produces swellings and pain at the wrist, ankle and sternal ends of ribs. Dental enamel shows thinning, pits and other defects.

Osteomalacia is characterized by poor intestinal absorption of calcium and phosphate, rise in urinary phosphate, negative calcium balance, muscular weakness, loss of bone calcium, softness, pain, deformities and easy fractures of bones, pelvic deformations, bending of vertebral column and bowed legs.

Both diseases, when caused by nutritional deficiency or failure of biosynthesis, may be cured by vitamin D. D₂, D₃, dihydrotachysterol and irradiated 7-dehydrositosterol are antirachitic in man.

A genetic deficiency of renal 25-HCC 1 α -hydroxylase depresses 1,25-DHCC formation. *Hereditary vitamin D-resistant rickets* results.

Chronic renal failure may depress 1,25-DHCC formation. This leads to *non-inherited vitamin D-resistant rickets or osteomalacia*.

Hypervitaminosis D: Continued excessive intake of vitamin D may cause loss of appetite, nausea, intense thirst, depression, irritability, excessive mobilization of bone calcium into blood, spontaneous fractures, hypercalcemia, muscle hypotonicity, metastatic calcification of soft tissues including cardiovascular, respiratory and renal tissues, abnormal bone development, poor body growth, high urinary calcium, blockage of kidney tubules by renal calculi and renal failure.

Functions: Vitamin D acts on target organs like bones, kidneys, intestinal mucosa and avian shell glands to regulate calcium and phosphate metabolisms. The principal active derivative of vitamin D is 1,25-DHCC. DeLuca and others have proposed that *vitamin D₃ is a prohormone* both synthesized in the body and obtained from food; it is converted to hormone-like 1,25-DHCC in the kidney under the inducing action of parathormone acting as a trophic hormone. Like other hormones, 1,25-DHCC is carried by blood to its target organs. In target organs like the intestine, 1,25-DHCC simulates steroid hormones in its *mode of action*. 1,25-DHCC enters the cytoplasm of the target cell and binds with a specific cytoplasmic protein (*mobile receptor*) which consequently undergoes a structural transformation and activation. This DHCC-receptor protein complex is translocated to the nucleus. There it probably binds to some nuclear protein bound

to specific genes, thereby increasing the transcription of mRNAs coding for specific enzymes and Ca^{2+} -binding or Ca^{2+} -carrier proteins. These proteins, whose syntheses are consequently enhanced, may hold Ca^{2+} in some tissues (e.g., the bone protein osteocalcin) or may participate in the active transport of Ca^{2+} across the cell membranes of intestinal, renal and bone cells.

1. *Vitamin D promotes intestinal absorptions of calcium and phosphate.* By influencing nuclear chromatin, 1,25-DHCC induces the synthesis of a Ca^{2+} -carrier protein (Ca^{2+} -ATPase) in intestinal cells. This protein increases Ca^{2+} absorption by actively transporting Ca^{2+} across the plasma membrane against electrochemical gradients. In rats and chicks, 1,25-DHCC also enhances intestinal phytase activity to promote Ca^{2+} absorption. Phosphate absorption is also enhanced probably through a gene-mediated induction of intestinal alkaline phosphatase. 25-HCC and 24,25-DHCC have lesser stimulating actions on Ca^{2+} absorption.

2. *Mineralisation of bones* is promoted by both 1,25-DHCC and 24,25-DHCC. The former is more than 30 times as effective as 25-HCC. 1,25-DHCC induces the synthesis of Ca^{2+} -binding proteins like osteocalcin and probably of alkaline phosphatase which increases phosphate ion concentration in the bone. Both these actions enhance mineral deposition in bones. 24,25-DHCC increases the synthesis and deposition of calcium hydroxyapatite in the bone. Vitamin D derivatives may also promote *bone resorption* and Ca^{2+} mobilization to raise the serum Ca^{2+} and Pi levels; this may result from an induction of Ca^{2+} -carrier proteins by 1,25-DHCC in osteoclasts, and an augmentation of the bone-resorbing effect of parathormone.

3. *Renal reabsorptions of Ca^{2+} and phosphate* are enhanced by 1,25-DHCC, leading to their retention in the body. In hypercalcemia

and hyperphosphatemia, synthesis of 1,25-DHCC is replaced by that of 24,25-DHCC, consequently lowering tubular reabsorptions of Ca^{2+} and phosphates as also their serum concentrations.

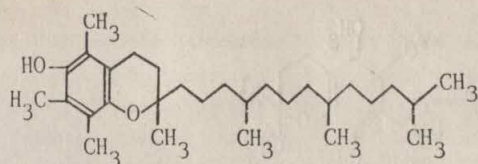
Assay: (a) *Biological method:* The healing effects of the standard vitamin preparation and of different doses of the test material on rachitic rats are compared by examining the zone of calcification at the ends of longitudinal sections of long bones like radius, ulna or tibia (*line test*). In another biological method, the effects of the test material and of the standard vitamin D preparation on the bone-ash content of growing chicks, are compared with each other. (b) *Chemical method:* Either a chloroform solution or an ethylene dichloride solution of a chromatographed extract of the test material is treated with antimony trichloride-acetyl chloride reagent and its light absorbancy is subsequently compared spectrophotometrically with that of a standard calciferol solution similarly treated.

7.13 VITAMIN E

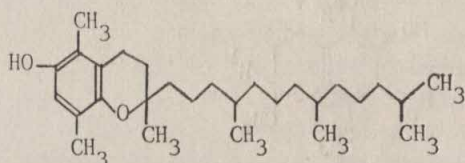
Sources: Vegetable oils like safflower oil, peanut oil, rapeseed oil, maize oil, wheat germ oil and soyabean oil, peanuts, almonds, sweet potato, lettuce, hydrogenated vegetable fats.

Daily requirements: Adult man: 10 mg; adult woman: 8 mg; pregnant woman: 10 mg; lactating woman: 11 mg; infants and children: 3-7 mg. The requirement rises with rising intakes of polyunsaturated fatty acids.

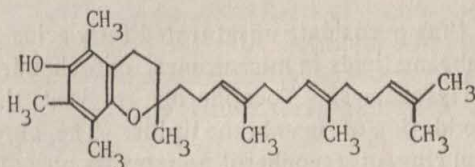
Chemistry: *Tocopherols* (e.g., α , β , γ and δ tocopherols) are pale yellow, viscous oils with vitamin E activity. They have a chromane nucleus with a phenolic OH group and an isoprenoid sidechain (Fig. 7.41). They are thermostable and soluble in fat-solvents like benzene, chloroform and ether, but are destroyed by oxidizing agents and ultraviolet rays. They are antioxidants.



α -Tocopherol
(5,7,8-trimethyltocol)



β -Tocopherol
(5,8-dimethyltocol)



α -Tocotrienol

Fig. 7.41. Some compounds with vitamin E activity.

Tocotrienols (e.g., α , β , γ and δ tocotrienols) are similar in structure to tocopherols except that they possess 3 double bonds in the sidechain. They show vitamin E activities.

Absorption : Vitamin E is absorbed from the small intestine, after incorporation into water-soluble micelles with the help of bile salts. Pancreatic lipases also help in its absorption. In obstructive jaundice, pancreatitis, sprue and steatorrhoea, failure of intestinal absorption of fat is accompanied by a failure of tocopherol absorption. The absorbed tocopherols are incorporated in chylomicron droplets which are released in the lymph. Reaching the liver, tocopherols are incorporated in lipoproteins and carried by blood to muscles and adipose tissues for storage.

Deficiency : In *male rats*, vitamin E deficiency produces loss of sperm motility,

degeneration of seminiferous tubules and sterility ; but Sertoli cells and interstitial cells of Leydig still continue thriving. In *female rats*, pregnancy may take place as oogenesis is not impaired, but the fetus dies and is resorbed from the uterus. Both sexes also show increased oxygen consumption of muscles, muscular dystrophy, atrophy of kidney tubules, creatinuria, hepatic necrosis, spasticity and paralysis.

In *calves, lambs and swine*, vitamin E deficiency leads to *muscular dystrophy (white muscle disease)*. It is characterized by fragmentation of fibres, loss of striations, hyaline degeneration, leucocytic infiltration and increased O_2 consumption of muscles, low muscle creatine and creatinuria. Histological lesions in the cardiac muscle, increased oxygen consumption of heart tissue and abnormal ECG are also observed (*cardiac myopathy*). Symptoms of muscular dystrophy show improvement on tocopherol administration.

Feeding of low-tocopherol diets to *premature infants* produces low plasma tocopherol, increased erythrocyte fragility, hemolytic or macrocytic anemia, extensive edema, reticulocytosis, thrombocytosis and intravascular thrombus formation ; increased susceptibility of RBC to hemolyzing effects of peroxides and dialuric acid is also noted in *adult human volunteers* on tocopherol-deficient diets. These symptoms are often aggravated on diets rich in polyunsaturated fatty acids. Clinical cases of tocopherol deficiency occur in sprue, obstructive jaundice, pancreatitis and steatorrhoea, where fats and fat-soluble vitamins fail to be absorbed ; increased susceptibility of RBC to hemolysis, weakness of muscles, creatinuria and spasticity of limbs are observed.

Hypervitaminosis E : Excessive intakes of vitamin E are reported to produce toxic symptoms such as headache, dizziness, fatigue, blurring of vision, dermatitis, acne, vasodilatation, gastrointestinal symptoms, prolonged

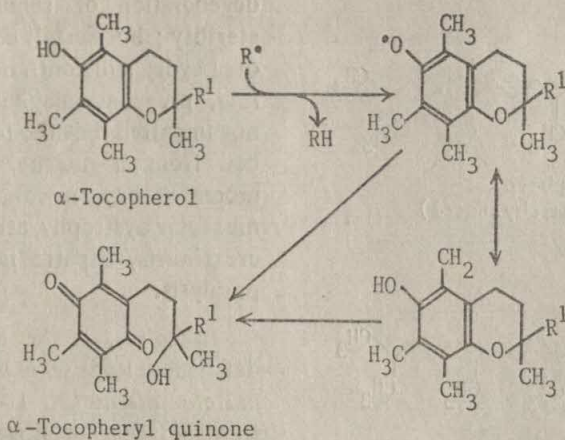


Fig. 7.42. α -Tocopherol as free radical scavenger. $\text{R}^\bullet$ =free radical.

clotting time and rise in serum cholesterol and fats.

Functions : Tocopherols act as antioxidants to prevent many undesirable oxidations in the body.

1. *Scavenger of free radicals :* By their antioxidant actions, tocopherols function as scavengers of free radicals to prevent their peroxidative effects on unsaturated lipids of membranes and thus help to stabilize membranes. Free radicals like free $\text{OH}^\bullet$ radical and superoxide anion (O_2^-) are formed during the action of some oxidoreductases such as the microsomal NADPH oxidase. Some toxic extrinsic materials like CCl_4 are also converted to free radicals like haloalkanes ($\text{CCl}_3^\bullet$) and free halogen radicals ($\text{Cl}^\bullet$). A free radical with an unpaired electron may take away hydrogen from a methylene group of a polyunsaturated fatty acid to change it to a fatty acid radical which binds with O_2 to give a fatty acid peroxy radical. The latter changes into a fatty acid hydroperoxide by accepting hydrogen from the methylene group of another polyunsaturated fatty acid, converting the latter into another fatty acid radical. These chain reactions form many fatty acid radicals and fatty acid hydroperoxides. Free radicals

may thus peroxidate unsaturated fatty acids of membrane lipids in microsomes, mitochondria and lysosomes. Tocopherols prevent this peroxidation of membrane lipids. The chromanol ring of tocopherol donates its phenolic hydrogen to reduce the free radical and is itself oxidized to the quinone form (Fig. 7.42). This antioxidant action of tocopherols on unsaturated membrane lipids is facilitated by the location of α -tocopherol in the membrane lipid bilayer. α -Tocopherol resides close to the polyunsaturated fatty acid tail of membrane phospholipids and to the membrane enzymes like NADPH-dependent mixed function oxidase which form free radicals. The isoprenoid sidechain of tocopherol remains bound by hydrophobic bonds to the nonpolar tail of arachidonyl residue of the membrane phospholipid while the methyl groups of the isoprenoid sidechain lie into pockets formed by bends of the fatty acid chain at its *cis* double bonds (Fig. 7.43). The chromane ring of tocopherol is oriented towards the polar head-groups of membrane phospholipids near the water-adjointing surface of the lipid layer. Located so close to both the membrane enzymes generating free radicals and the polyunsaturated fatty acids, tocopherols can best prevent peroxidation of those fatty

acids by reducing free radicals. In this way, tocopherols preserve the structural and functional integrities of membrane-bound organelles, and thereby prevent muscular dystrophy, hepatic necrosis or increased erythrocyte fragility. The increased O_2 consumption by dystrophic muscles also supports the antioxidant role of tocopherols. E-deficiency promotes peroxidation of polyunsaturated fatty acids in membrane phospholipids of lysosomes; lysosomal hydrolases consequently leak out to cause extensive damages in dystrophy. Selenium may prevent some E-deficiency symptoms like muscular dystrophy (vide 6.13).

2. Antioxidant action of tocopherols prevents the peroxidative effects of atmospheric O_3 , H_2O_2 and NO_2 on membrane lipids of bronchi, bronchioles and pulmonary alveoli. Thus, tocopherols reduce toxic effects of air-pollutants.

3. Antioxidant action of tocopherol, located close to the integral membrane proteins containing selenide and nonheme iron, helps to protect selenide at the active sites of those proteins against the effects of free radicals.

4. By preventing peroxidative changes in mitochondrial membranes, tocopherols maintain the translocation of phosphate ions into mitochondria and may thereby enhance oxidative phosphorylation. Tocopherols may also

prevent oxidation of sulfhydryl enzymes participating in mitochondrial oxidations.

5. *Antisterility factor*: Tocopherols prevent sterility in lower animals like rodents. As other antioxidants like methylene blue may also prevent reproductive failures in E-deficient rats, the antisterility effect may be related to the antioxidant action of vitamin E.

Assay: 1. *Physicochemical methods*:

(a) In a *colorimetric method*, both a solvent extract of the unknown sample and a standard tocopherol solution are treated with $FeCl_3$ and either α, α' -dipyridine or bathophenanthroline. Tocopherols reduce Fe^{3+} to Fe^{2+} which forms a colored complex with dipyridine or bathophenanthroline. The color produced in each of the two solutions is measured colorimetrically. (b) A *spectrophotometric method* involves the measurement of extinctions of an ethanol extract of the unknown sample and a standard tocopherol solution at specific wavelengths. (c) In a *spectrofluorometric method*, free tocopherols are estimated in a sample by measuring the fluorescence of its solvent extract and of a standard solution at 295 and 340 nm; tocopheryl esters have to be cleaved into free tocopherols for emitting fluorescence.

2. *Biological methods*. (a) In the *fetal resorption test*, comparisons are made between the effects of the test material and of the

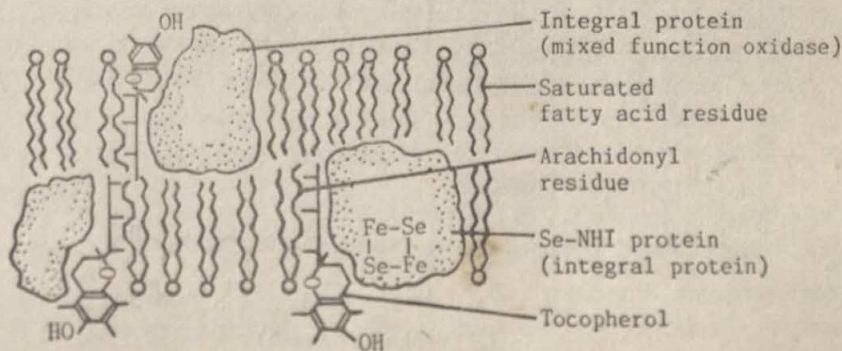


Fig. 7.43. Tocopherol in membrane lipid bilayer.

standard vitamin preparation in preventing death and resorption of fetus in the uterus of tocopherol-deficient female rats up to 19 days after confirmation of pregnancy.

(b) In the *muscular dystrophy test*, rates of cure of either thigh muscle dystrophy or creatinuria are estimated in tocopherol-deficient rats following administration of the unknown sample and a standard vitamin E preparation. (c) *Erythrocyte hemolysis test* compares the effects of administration of the unknown sample and a standard preparation in preventing hemolysis by dialuric acid in groups of E-deficient rats. (d) A clinical *hemolysis test for vitamin E deficiency* in man consists of an estimation of the susceptibility of erythrocytes to hemolysis on treatment with a hydrogen peroxide solution.

7.14 VITAMIN K

Sources : Egg-yolk, liver, green leaves of spinach, ipomea, lettuce and cabbage, vegetable oils, sun-dried fish, wheat bran and soyabean. Ordinarily, sufficient amounts are synthesized by intestinal bacteria and absorbed from the intestine. So, normal adults may not require the vitamin in the food.

Chemistry : Vitamins K are fat-soluble naphthoquinones with polyisoprenoid side-chains. Vitamin K₁ or phyloquinone or 2-methyl-3-phytyl-1,4-naphthoquinone is a light yellow oil found in dark-green leaves and vegetable oils (Fig. 7.44). Vitamins K₂ or menaquinones occur in fish-meal and are also synthesized by intestinal microflora; e.g., 2-methyl-3-difarnesyl-1,4-naphthoquinone (a yellow solid melting at 54°C) and 2-methyl-3-hexaprenyl-1,4-naphthoquinone. Vitamins K absorb ultraviolet rays and are thermostable, photolabile and alkali-labile. Menadione or vitamin K₃ or 2-methyl-1,4-naphthoquinone is a synthetic product with vitamin K activity.

Absorption : Vitamin K is absorbed from the small intestine, after incorporation into water-soluble micelles with the help of bile salts. Failure of bile flow into the intestine due to a bile fistula or a clinical obstruction of bile ducts may produce vitamin K deficiency with a resulting fall in the plasma prothrombin level. In pancreatitis, sprue and steatorrhoea, the failure of intestinal absorption of fat is accompanied by a failure of vitamin K absorption.

Deficiency : Vitamin K deficiency can be produced in rats and chicks by K-deficient

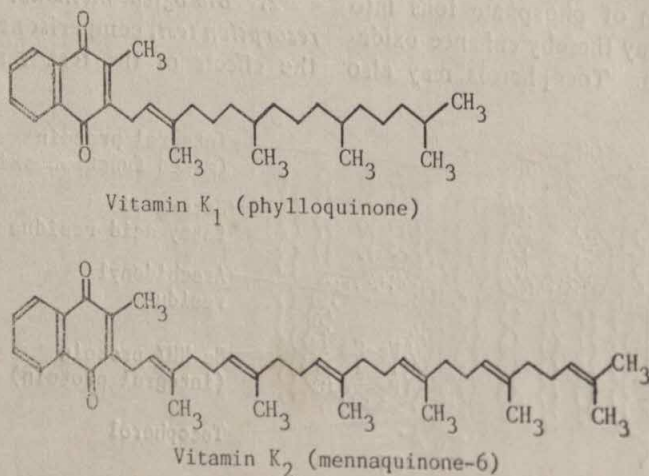


Fig. 7.44. Two compounds with vitamin K activity.

diets along with sulfa drugs to kill the intestinal flora. Vitamin K deficiency leads to a fall in plasma prothrombin level, an abnormally long coagulation time and a tendency to spontaneous hemorrhages.

Cattle, fed with spoilt sweet clover hay, develop a bleeding disease with low prothrombin, proconvertin and Stuart factor activities, fall in O_2 consumption, poor oxidative phosphorylation and prolonged coagulation time. This *sweet clover disease* is caused by the action of dicumarol, vitamin K-antagonist present in spoilt sweet clover hay. *Anti-vitamins K* such as dicumarol, phenindione and warfarin are used for lowering plasma prothrombin in thrombosis cases.

In the first few days of post-natal life, some human infants suffer from *hypoprothrombinemia* and bleedings in many tissues, including the central nervous system, intestines and adrenals, because little vitamin K can pass from the mother to the fetus through placental membranes and also because intestinal microflora has not been established yet. As intestinal bacteria begin to synthesize the vitamin, blood prothrombin gradually rises and the disorder subsides. K-deficiency sometimes occurs in adults due to intestinal malabsorption in sprue, obstructive jaundice and coeliac disease. K-deficiency may also result from a failure of microbial synthesis after prolonged administration of sulfa drugs or antibiotics. Symptoms include poor activities of prothrombin and proconvertin in plasma, a prolonged coagulation time and bleeding.

Hypervitaminosis K: Injections of large doses of vitamin K may produce jaundice in human infants.

Functions :

1. Vitamin K promotes blood coagulation by helping in the post-translational modification of some blood coagulation proteins such as prothrombin and Stuart factor. The natural

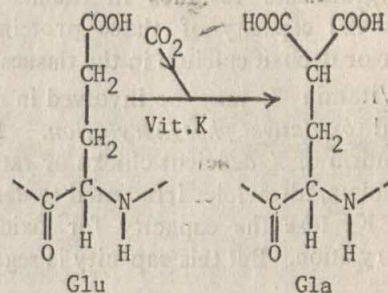


Fig. 7.45. Conversion of Glu residue of prothrombin to Gla residue.

quinone form of vitamin K is first reduced to its hydroquinone form in the liver microsomes by a dehydrogenase and NADPH. Vitamin K hydroquinone functions as a coenzyme for a microsomal carboxylase, using CO_2 to incorporate an additional $COOH$ group at the γ -C of specific glutamate residues of specific coagulation proteins like prothrombin (Fig. 7.45). This changes the glutamate residues (Glu) to γ -carboxyglutamates (Gla). Possibly the K-hydroquinone may simultaneously change into 2,3-epoxide which is reduced back to the quinone form by microsomal epoxide reductase. Dicumarol inhibits epoxide reductase to block this cyclic change of vitamin K. All of the first ten Glu residues in the N-terminal part of prothrombin are carboxylated to Gla residues with the help of vitamin K. These Gla residues provide Ca^{2+} -binding sites in the N-terminal segment of prothrombin. Ca^{2+} -ions, chelated to these Gla residues, anchor prothrombin, activated Stuart factor and accelerin close together to the phospholipid membranes of disintegrated platelets. This enhances the rate of activation of prothrombin by many thousand times to promote blood coagulation.

2. Vitamin K similarly helps to carboxylate specific glutamate residues in Ca^{2+} -binding proteins in many tissues like bones, spleen, placenta and kidneys to generate

8. DIGESTION AND ABSORPTION OF FOOD

For utilization in the body, food constituents have to pass through the intestinal epithelium from the intestinal lumen to blood or lymph. This absorption of food is carried out either by energy-dependent active processes or by physical processes such as diffusion and osmosis which depend on concentration gradients.

Before they may be absorbed, bigger or less soluble molecules of food are digested into smaller and more soluble molecules. Enzymes of digestive juices carry out *extracellular digestion* in the gastrointestinal lumen. Some partly digested materials may also undergo *intracellular digestion* by enzymes inside intestinal epithelial cells. Digestion consists mainly of hydrolysis and in a few cases, phosphorylisis.

Saliva helps mainly in lubricating the food with mucin for mastication and swallowing. Salivary water also dissolves soluble food constituents. In addition, ptyalin of human and porcine saliva initiates the digestion of starch in the mouth. *Gastric juice* carries out digestion of proteins by its enzymes and HCl. *Bile* helps principally in the digestion and absorption of lipids with its organic bile salts. *Pancreatic juice* contains several enzymes for digesting proteins, nucleic acids, carbohydrates and lipids. *Intestinal juice* has several enzymes for completing the digestion of proteins, nucleic acids, carbohydrates and lipids. Compositions of these juices are given in Table 8.1. In addition, large intestine and ruminant stomach harbour numerous microorganisms for digesting plant fibres into products utilized by the host.

8.1 DIGESTION OF CARBOHYDRATES

Starch forms more than 50% of the carbohydrates in human food. Smaller amounts of

dextrins, disaccharides and monosaccharides also occur in food. Cellulose normally forms the major bulk of food carbohydrates in many herbivores but only a minor fraction of human food. Salivary, pancreatic and intestinal carbohydrases hydrolyze specific glycoside linkages in polysaccharides, oligosaccharides and disaccharides. But in the absence of any cellulose-digesting enzyme in higher animals, they have to depend on microbial digestion of cellulose in large intestine or ruminant stomach.

1. **Digestion by saliva:** Starch is digested primarily by amylases of saliva and pancreatic juice. Both the salivary amylase (ptyalin) and the pancreatic amylase are α -amylases or dextrinogenic amylases. α -Amylases are *endoamylases*, i.e., they can hydrolyze α -1,4 glycosidic linkages at random deep inside the starch molecule (Fig. 8.1) and even between the α -1,6 glycosidic linkages forming the branching points of the molecule. But they cannot hydrolyze the α -1,6 linkages, β -1,4 linkages, α -1,4 linkages immediately near α -1,6 linkages, and α -1,4 linkages in disaccharides. α -Amylases digest starch mainly into maltose and α -limit dextrins and to a smaller extent, maltotriose and isomaltose. α -Limit dextrins are branched oligosaccharides of 4-8 glucose residues, have low molecular weights and cannot be hydrolyzed further by

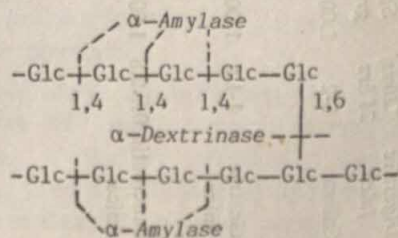


Fig. 8.1. Glycoside linkages hydrolyzed by α -amylases and α -dextrinase.

TABLE 8.1. Composition of digestive juices.

Digestive juice	Litres/24 hrs	Specific gravity	pH	Water %	Minerals		Organic constituents		
					%	Constituents	%	Enzymes	Others
Saliva	1-2	1.003-1.010	5.9-7.2	99.5	0.2	Na ⁺ , Cl ⁻ , HCO ₃ ⁻ , K ⁺ , Ca ²⁺ , Mg ²⁺ , HPO ₄ ²⁻ , SCN ⁻	0.3	Ptyalin, lysozyme	Mucin, urea, lactate
Gastric juice	1-1.5	1.002-1.004	0.9-1.2	99.3	0.5 0.15	HCl Na ⁺ , Cl ⁻ , K ⁺ , Ca ²⁺ , Mg ²⁺ , HPO ₄ ²⁻ , H ₂ PO ₄ ⁻	0.4	Pepsin, rennin, lipase, intrinsic factor, lysozyme	Mucin, lactate
Pancreatic juice	0.7-0.8	1.006-1.014	7.1-8.4	98.6	0.9	Na ⁺ , HCO ₃ ⁻ , Cl ⁻ , K ⁺ , Ca ²⁺ , HPO ₄ ²⁻	0.5	Trypsin, chymotrypsin, carboxypeptidases, elastase, pancreatic amylase, pancreatic lipase, phospholipase, cholesterol esterase, RNase, DNase	—
Intestinal juice	1.2-1.8	1.008-1.012	6.4-9.1	98.4	0.8	Na ⁺ , HCO ₃ ⁻ , Cl ⁻ , K ⁺ , Ca ²⁺ , HPO ₄ ²⁻	0.8	Enteropeptidase, aminopeptidases, dipeptidases, amylase, sucrase, lactase, maltase, α -dextrinase, trehalase, lipase, lecithinase, lysolecithinase, phosphatases, polynucleotidase, nucleotidases, nucleoside phosphorylases	Mucin
Bile (hepatic)	0.5-1	1.008-1.012	6.9-8.6	97.4	0.8	Na ⁺ , HCO ₃ ⁻ , Cl ⁻ , K ⁺ , Ca ²⁺ , HPO ₄ ²⁻	1.8	—	Bile salts (1.2%), mucin & bile pigments (0.7%), cholesterol (0.1%), fats, soaps, lipids
Bile (bladder)	0.2-0.5	1.032-1.040	5.6-7.6	88.8	0.9	Na ⁺ , HCO ₃ ⁻ , Cl ⁻ , K ⁺ , Ca ²⁺ , HPO ₄ ²⁻	10.3	—	Bile salts (5.4%), mucin & bile pigments (3.5%), cholesterol (0.6%), fats, soaps, lipids

α -amylases due to the abundance of α -1,6 linkages; maltotriose is a trisaccharide of glucose while isomaltose is a maltose with its glucose molecules linked by an α -1,6 linkage instead of an α -1,4 linkage. All these products of α -amylase action have the hemiacetal group at the reducing end of the molecule in the α configuration. α -Amylases contain firmly bound Ca^{2+} whose removal by dialysis affects the enzyme activity adversely. Cl^- activates α -amylases and changes their pH-activity curves favourably, but does not seem to affect their K_m values. Cooking of food facilitates α -amylase action by breaching the cellulosic cell wall, because α -amylases cannot digest cellulose, containing β -1,4 linkages, and consequently cannot enter through the cell wall to digest starch stored in plant cells.

Ptyalin, the salivary α -amylase, starts digesting starch in the mouth where salivary HCO_3^- maintains the pH near 6.6-6.8 for optimum ptyalin activity. It hydrolyzes starch into progressively smaller dextrans—erythrodestrins (colored red with iodine), then achrodestrin (no color with iodine) and finally α -limit dextrans are formed (Fig. 8.2). Branched amylopectin part of starch is changed by ptyalin to maltose, α -dextrans, maltotriose and isomaltose. But unbranched amylose part, devoid of α -1,6 linkage, is hydrolyzed to maltose and maltotriose only. Ptyalin action continues for some time at the centre of the food bolus in stomach also. Ultimately, the fall in pH to 3 or less due to gastric HCl denatures ptyalin and stops its further action.

[Many plant materials such as wheat, barley, soyabean, sweet potato and malt contain β -amylases. Unlike α -amylases, β -amylases are saccharogenic amylases and *exoamylases*. They hydrolyze α -1,4 glycosidic linkages of starch at its nonreducing end to produce successively many molecules of β -maltose and leaves some large molecules of β -limit dextrans having high MW. Both the

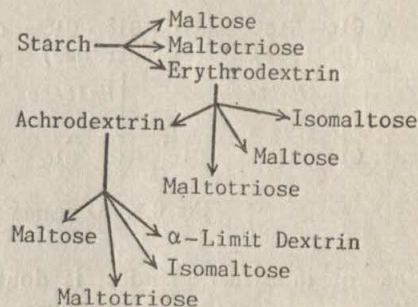


Fig. 8.2. Starch digestion by α -amylases.

products bear the hemiacetal group at the reducing end of the chain in the β configuration because of an inversion of α to β configuration during the hydrolysis of starch by β -amylases. β -Amylases cannot hydrolyze α -1,4 linkages between two α -1,6 linkages in the molecular chain; so, they leave much larger limit dextrans undigested, compared to those left by α -amylases. β -Amylases do not possess firmly bound Ca^{2+} . γ -Amylases or glucamylases of plants are also saccharogenic *exoamylases*. But unlike β -amylases, they hydrolyze terminal α -1,4 linkages to liberate successive molecules of glucose from starch.]

2. Digestion in stomach: Gastric HCl can hydrolyze some sucrose into glucose and fructose. Besides, swallowed ptyalin continues to digest starch for some time in stomach.

3. Digestion by pancreatic juice: Pancreatic juice contains an α -amylase called pancreatic amylase which hydrolyzes α -1,4 linkages in starch at pH 6.9-7.2 in presence of Cl^- to produce maltose, α -limit dextrans, isomaltose and maltotriose in the same way as salivary amylase.

4. Digestion by intestinal juice: Intestinal juice and mucosa contain several carbohydrases. *Intestinal amylase*, found in intestinal mucosa extract, may hydrolyze α -1,4 linkages in starch to produce maltose, α -limit dextrans, isomaltose and maltotriose. But its activity is low and its real source, whether

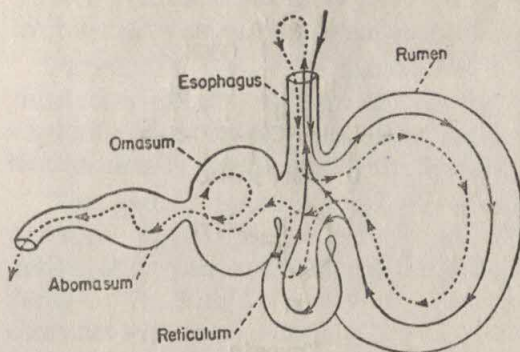


Fig. 8.4. Course of food in four chambers of ruminant stomach before (solid line) and after (broken line) rumination.

HCO_3^- -rich saliva are secreted and swallowed into the rumen where the saliva maintains an optimal pH for microbial growth by neutralizing organic acids produced by fermentation. This ensures a far higher and more varied bacterial population in rumen and reticulum than in the large intestine of nonruminants.

8.2 DIGESTION OF PROTEINS

Proteins are digested in the alimentary canal by proteases or peptidases which hydrolyze specific peptide bonds in their substrates. Proteases have frequently been classified into two types: (a) *Exopeptidases* such as carboxypeptidases and aminopeptidases hydrolyze only a terminal peptide bond connected to either the C-terminal or the N-terminal amino acid residue of the peptide chain and release that end-amino acid free. (b) *Endopeptidases* such as pepsin, trypsin, enteropeptidase and chymotrypsin hydrolyze specific peptide bonds even deep in the middle of the peptide chain to cleave the peptide into more than one smaller peptide.

Some specific group or residue, occurring at the active site of a protease, is essential for its activity. Proteases are classified into families according to the nature of such critical groups or residues at their active sites.

(a) *Serine proteases* such as trypsin, chymotrypsin and elastase possess a critical serine residue at the active site. They are irreversibly inhibited by diisopropylphosphoryl-fluoridate (DIPF)—the latter binds covalently with the sidechain CH_2OH of the critical serine residue to form an inactive and stable diisopropylphosphoryl-enzyme complex. (b) *Thiol proteases* such as papain of papaya fruit possess at the active site a critical cysteine residue whose sidechain SH must remain free for their activity. They are irreversibly inhibited by iodoacetamide which affects the SH group. (c) *Zinc proteases* such as carboxypeptidase A must contain a tightly bound Zn^{2+} at the active site for activity. (d) *Carboxyl proteases* or *acid proteases* such as pepsin must possess at the active site a critical dicarboxylic amino acid residue like an aspartate with a sidechain COOH . They mostly have optimum pHs in the acidic range.

To prevent proteolysis of cellular or extracellular components, many proteases (e.g., pepsin, trypsin, chymotrypsin, rennin, elastase and carboxypeptidases) are synthesized as inactive *proenzymes* or *zymogens*, and stored in membrane-bound secretory granules. On reaching the site of action after secretion, the proenzyme is irreversibly hydrolyzed by either pH changes or specific proteases into the active enzyme and one or more inactive peptides (*irreversible covalent activation*).

1. *Digestion in stomach*: Protein digestion is started in the stomach with the help of HCl and two proteases (pepsin and rennin) of the gastric juice. In ruminants, gastric juice is secreted and peptic digestion of protein is carried out in *abomasum*, the fourth or last chamber of their stomach. In birds, gastric juice containing HCl and pepsin is secreted in *proventriculus*, the tubular first chamber of their two-chambered stomach.

(a) *Action of pepsin and HCl*: Pepsin occurs in the gastric juice of fish, amphibians,

reptiles, birds and mammals, but not in invertebrates. It is secreted in the gastric juice as an inactive proenzyme called *pepsinogen*. The latter undergoes irreversible covalent activation to pepsin in the gastric lumen either spontaneously at a pH of 2 or below, provided by gastric HCl, or by the autocatalytic action of already activated pepsin at a pH of about 4.6. During the activation, hydrolysis of a specific peptide bond in pepsinogen releases a 44-residue inactive peptide from its N-terminal end and exposes the sidechain COOH of an active-site aspartate residue in the remaining peptide chain, converting the latter to pepsin. Human fundic and pyloric mucosae carry immunologically distinct pepsinogens I and II respectively.

Pepsin is a *carboxyl protease* carrying two reactive aspartate residues at its active site. For activity, one of the critical aspartate residues must bear an ionized sidechain COO^- while the other must have a nonionized sidechain COOH—these are maintained only within the pH range of 1.6-3.2. So, like most carboxyl proteases, pepsin is an acid protease, its optimum pH varying between 1.6 and 3.2 according to the substrate.

Gastric HCl helps in peptic digestion in three ways. (i) It lowers the gastric pH to 2 or below to activate pepsinogen to pepsin. (ii) It maintains the gastric pH at 1.6-3.2 to ensure maximum pepsin activity. (iii) HCl denatures some native proteins to *acid metaproteins* by uncoiling their peptide chains through the disruption of the coil-stabilizing electrostatic and hydrogen bonds. This facilitates pepsin action on proteins.

Pepsin is an endopeptidase. It hydrolyzes peptide bonds even deep in the middle of the peptide chain to digest native proteins, coagulated proteins and acid metaproteins to proteoses and peptones. It acts specifically on peptide bonds between the $\alpha\text{-COOH}$ of an aromatic amino acid (phenylalanine, tyrosine

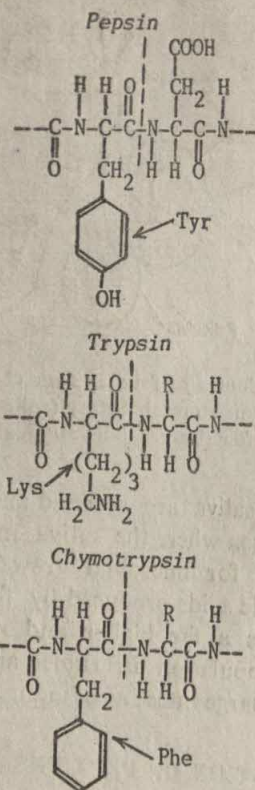


Fig. 8.5. Examples of peptide bonds hydrolyzed by pepsin, trypsin and chymotrypsin.

or tryptophan), methionine or leucine and the $\alpha\text{-NH}_2$ of either a dicarboxylic or an aromatic amino acid (Fig. 8.5). So, it cannot hydrolyze protamines deficient in aromatic amino acids. Like other proteases of alimentary canal, pepsin cannot digest keratin because of a close-packing of its peptide chains. But pepsin is very effective in solubilizing gelatins and in digesting collagens which resist the action of many other proteases.

Pepsin can also coagulate milk. It hydrolyzes peptide bonds involving aromatic amino acid residues in the soluble phosphoprotein casein of milk, cleaving it to two soluble proteins, viz., paracasein and a protease-like whey protein (Fig. 8.6). Paracasein reacts spontaneously with Ca^{2+} to get precipitated as

calcium paracaseinate (curd) which is further hydrolyzed by pepsin and other proteases.

Action of pepsin is terminated in the duodenum due to its denaturation by the alkalinity of pancreatic juice and bile, and its digestion by trypsin at the alkaline pH.

The gastric mucosa itself is normally protected against the corrosive proteolytic action of pepsin and HCl by the following factors. (i) Soluble mucin of gastric juice combines with some gastric HCl to form less ionizable complexes. (ii) The thick mucin layer, gelled by HCl on the mucosal surface, provides a mechanical barrier against the contact of HCl and pepsin with the mucosa. (iii) Mucosal cell membrane is significantly impermeable to HCl and pepsin. (iv) Activation of pepsinogen is postponed till after its secretion into the gastric contents.

(b) *Action of rennin*: An inactive pro-enzyme called *prorennin* occurs in the mucous membrane of abomasum and the gastric juice of calf. It is spontaneously hydrolyzed at the gastric pH provided by HCl to yield an inactive peptide and an active protease called rennin. Rennin is a milk coagulating enzyme (Fig. 8.6). It hydrolyzes peptide bonds involving α -COOH of aromatic amino acids in casein to cleave it to whey protein and paracasein, the latter getting precipitated as calcium paracaseinate (curd). But rennin is absent in man and in adult cow.

2. Digestion by pancreatic juice: Pancreatic juice contains five proteases, viz., trypsin, chymotrypsin, elastase and carboxypeptidases A and B. All five are secreted as inactive proenzymes and get activated in the intestinal lumen. As will be described below, trypsin is activated by *enteropeptidase* of intestinal juice and in turn activates the other proenzymes as well as the remaining inactive trypsin molecules. Thus, all the pancreatic proteases are simultaneously activated in a coordinated manner, triggered by enteropeptidase. This ensures the concurrent action

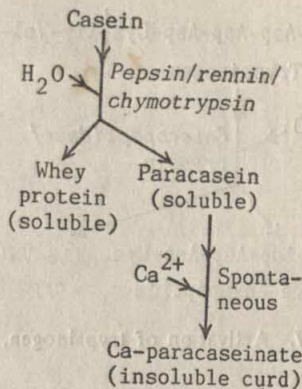


Fig. 8.6. Action of milk coagulating enzymes.

of all these proteases on different peptide bonds in a protein for its rapid and complete digestion.

Pancreatic tissue is effectively protected against its own proteases because they are all secreted as inactive proenzymes and stored in membrane-enclosed secretory granules. Moreover, pancreas secretes a *trypsin inhibitor protein* (MW $\approx$ 6 kdal) which possesses close structural complementarity with the active site of trypsin and binds tightly with it to form a highly stable enzyme-inhibitor complex. This precludes the possibility of premature activation of pancreatic proteases as well as phospholipase A_2 by any trypsin activated accidentally in pancreas itself. Premature activation of these pancreatic enzymes may be a factor in causing *acute pancreatitis* with severe and fatal destruction of pancreatic tissue.

(a) *Action of trypsin*: Trypsin is secreted as inactive *trypsinogen*. In the intestinal lumen, trypsinogen is hydrolyzed to active trypsin by enteropeptidase (enterokinase) of intestinal juice when the intestinal contents have a pH 5.2-6.0. But with the progressive rise in intestinal pH to 7.9, already activated trypsin hydrolyzes more trypsinogen molecules to trypsin. Both enteropeptidase and trypsin hydrolyze the peptide bond between the

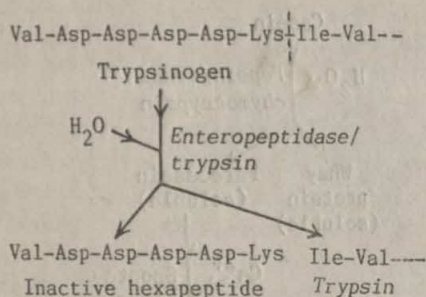


Fig. 8.7. Activation of trypsinogen.

α -COOH of lysine⁶ and the α -NH₂ of isoleucine⁷ (positions counted from the N-terminal end) in trypsinogen to release an inactive hexapeptide from its N-terminal end, the rest of the molecule becoming active trypsin (Fig. 8.7).

Trypsin is a serine protease and endopeptidase. Its optimum pH ranges nearly 7-9 according to the substrate. It hydrolyzes specifically the peptide bonds involving the α -COOH of basic amino acid residues (arginine or lysine) deep inside a peptide chain (Fig. 8.5). It thus digests proteins (particularly basic proteins like protamines and histones) into peptides.

Trypsin also helps in protein digestion by activating inactive chymotrypsinogen, procarboxypeptidases A and B, proelastase and even trypsinogen itself to the respective active proteases (see below).

Trypsin activates phospholipase A₂ of pancreatic juice to phospholipase A₂ (vide 8.3).

Trypsin has very little action on casein and does not coagulate milk. But it can coagulate blood—it hydrolyzes four arginyl-glycine (Arg-Gly) peptide bonds, one in each of four peptide chains of fibrinogen, to release four inactive fibrinopeptides from the N-terminal ends. What is left of fibrinogen is a fibrin monomer which changes to fibrin polymer of blood clot.

(b) *Action of chymotrypsin:* Chymotrypsin is secreted as inactive chymotrypsinogen. In the intestinal lumen, trypsin hydrolyzes the peptide bond between the α -COOH of arginine¹⁵ and the α -NH₂ of isoleucine¹⁶ in chymotrypsinogen to release a 15-residue inactive peptide from the N-terminal end of the latter (Fig. 8.8). This changes chymotrypsinogen to active π -chymotrypsin. Each π -chymotrypsin molecule hydrolyzes many other molecules of π -chymotrypsin to δ -chymotrypsin with the release of an inactive serylarginine (Ser-Arg) dipeptide. π -Chymotrypsin next hydrolyzes δ -chymotrypsin to active and stable α -chymotrypsin with the release of an inactive threonylasparagine (Thr-Asn) dipeptide.

Both π - and α -chymotrypsins (MW $\approx$ 25 kdal) are active forms of the enzyme. They are endopeptidases belonging to the family of serine proteases. They possess pH optima of 7-8. Chymotrypsins digest proteins and peptides to smaller peptides by hydrolyzing peptide bonds connected with the α -COOH of either aromatic amino acids (Phe, Tyr or Trp) or amino acids with large nonpolar sidechains

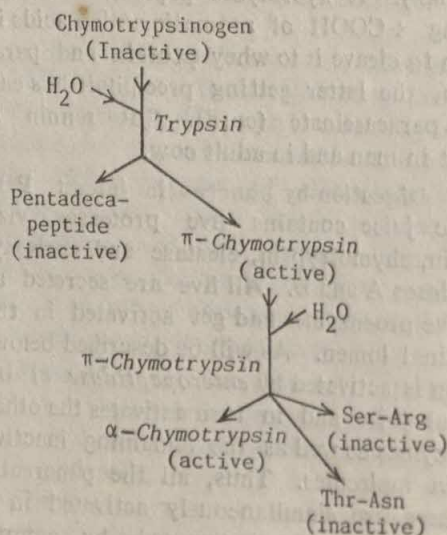


Fig. 8.8. Activation of chymotrypsinogen.

(Met, Leu, etc.) (Fig. 8.5). They are milk coagulating enzymes in particular. Like pepsin and rennin, they hydrolyze casein to paracasein, leading to the formation of curd (Fig. 8.6).

(c) *Action of elastase* : Elastase is secreted as inactive proelastase which is hydrolyzed by trypsin to give the active enzyme and an inactive peptide. Elastase is a serine protease and endopeptidase. It hydrolyzes specifically peptide bonds involving neutral aliphatic amino acids with small uncharged sidechains. It digests particularly elastin of yellow elastic fibres to peptides.

(d) *Action of carboxypeptidases* : Two carboxypeptidases have been identified in the pancreatic juice. Both are exopeptidases, secreted as inactive procarboxypeptidases in the pancreatic juice and activated by trypsin. Both enzymes are ineffective in hydrolyzing dipeptides. Both have pH maxima of about 7.5-8. (i) *Carboxypeptidase A* is a zinc protease. Trypsin degrades the subunit III of procarboxypeptidase A and changes the subunit II to an active protease; the latter then acts with trypsin on the subunit I to

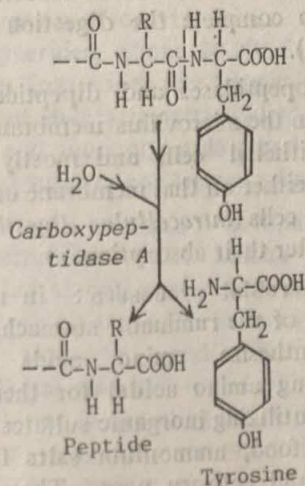


Fig. 8.9. Example of C-terminal peptide bonds hydrolyzed by carboxypeptidase A.

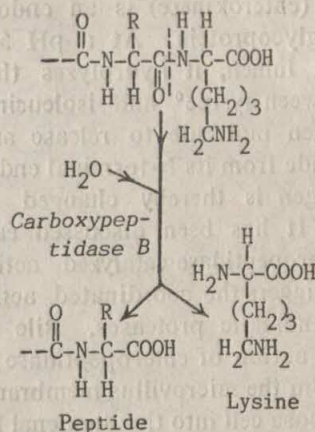


Fig. 8.10. Example of C-terminal peptide bond hydrolyzed by carboxypeptidase B.

change it to active carboxypeptidase A. Carboxypeptidase A can hydrolyze only the terminal peptide bond connected to the C-terminal amino acid residue, particularly if the latter is an aromatic (A) amino acid (Phe, Tyr or Trp) or an aliphatic amino acid with branched sidechain (Val, Leu or Ile) (Fig. 8.9); but it cannot hydrolyze the terminal peptide bond connected with C-terminal arginine, lysine or proline. Its action releases the C-terminal amino acid in the free form, shortening the peptide by one amino acid residue. (ii) *Carboxypeptidase B* is formed by the activation of inactive procarboxypeptidase B by trypsin. Unlike carboxypeptidase A, carboxypeptidase B hydrolyzes terminal peptide bonds connected with basic (B) amino acids (Arg, Lys) at the C-terminal end of the peptide (Fig. 8.10).

3. *Digestion by intestinal juice* : Intestinal proteases include enteropeptidase, aminopeptidases and dipeptidases. Except enteropeptidase, others are required to hydrolyze small peptides produced by gastric and pancreatic digestion. So, except enteropeptidase, they are exopeptidases hydrolyzing only terminal peptide bonds to release only free amino acids from the peptide.

(a) *Action of enteropeptidase*: Enteropeptidase (enterokinase) is an endopeptidase. It is a glycoprotein. At a pH 5.2-6 in the intestinal lumen, it hydrolyzes the peptide bond between lysine⁶ and isoleucine⁷ in the trypsinogen molecule to release an inactive hexapeptide from its N-terminal end (Fig. 8.7). Trypsinogen is thereby changed to active trypsin. It has been discussed earlier how this enteropeptidase-catalyzed activation of trypsin triggers the coordinated activation of several pancreatic proteases. Bile salts may help the action of enteropeptidase by liberating it from the microvillus membrane of duodenal mucosa cell into the duodenal lumen.

(b) *Action of aminopeptidases*: They hydrolyze the terminal peptide bond connected to the N-terminal amino acid residue of a peptide chain to release that amino acid from the peptide. Successive actions of aminopeptidases remove the N-terminal amino acid at each step, changing the peptide stepwise into a dipeptide. But aminopeptidases cannot hydrolyze dipeptides. *Leucine aminopeptidase* hydrolyzes the N-terminal peptide bond connected to a leucine residue as the end-amino acid and releases the N-terminal leucine from the peptide

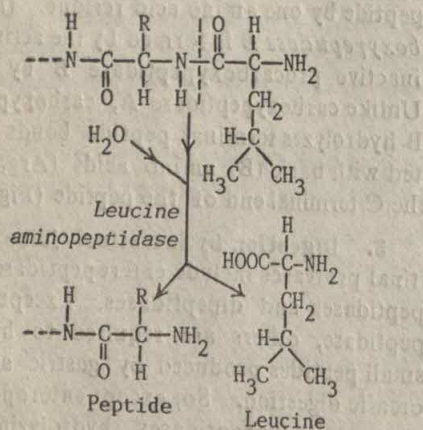


Fig. 8.11. N-terminal peptide bond hydrolyzed by leucine aminopeptidase.

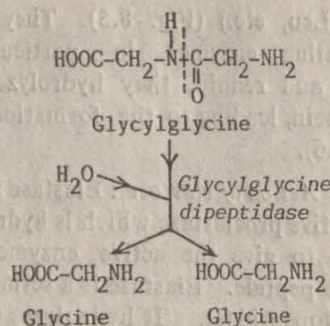


Fig. 8.12. Hydrolysis of the dipeptide glycylglycine by glycylglycine dipeptidase.

(Fig. 8.11). It can also hydrolyze to some extent the peptide bonds connected with other N-terminal amino acids with aliphatic side-chains, but not proline. It requires Zn^{2+} , Mn^{2+} or Mg^{2+} for catalysis. *Proline aminopeptidase* hydrolyzes specifically the peptide bond connected with the N-terminal proline residue of a peptide to release that proline from the peptide. It may play a significant role in the digestion of prolyl peptides formed by the partial digestion of collagens and prolamines.

(c) *Action of dipeptidases*: Dipeptidases such as glycylglycine dipeptidase hydrolyze dipeptides, each into two molecules of amino acids, to complete the digestion of proteins (Fig. 8.12).

Aminopeptidases and dipeptidases occur mainly on the microvillus membrane of intestinal epithelial cells and mostly hydrolyze peptides either on that membrane or inside the intestinal cells (*intracellular digestion*) immediately after their absorption.

4. *Microbial processes*: In rumen and reticulum of the ruminant stomach, bacterial flora synthesize amino acids (including S-containing amino acids) for their cellular proteins, utilizing inorganic sulfates and phosphates of food, ammonium salts fed to the animal, and salivary urea. These bacterial proteins are subsequently digested by proteases of the host in the abomasum and small

intestine to yield amino acids which are then absorbed. Even essential amino acids may be thus obtained by ruminants from microbial sources. Colonic and cecal bacteria of nonruminants may also synthesize amino acids and proteins in similar ways. But large intestine is located at the hind end of the alimentary canal and secretes no protease either. So, much of the bacterial proteins synthesized in the large intestine fails to be utilized by the animal and is lost with feces. Indeed, most of the human fecal nitrogen comes from bacterial sources. Still, amino acids synthesized by colonic bacteria may gain nutritional significance when on a low-protein diet, as in people thriving on sweet potato.

8.3 DIGESTION OF LIPIDS

Fats, phospholipids and sterol esters are digested by the hydrolysis of their ester linkages by esterases of the alimentary system. Bile salts play essential roles in the digestion of lipids.

According to the *partition theory* of fat digestion and absorption, the entire amount of food fat need not be digested completely before absorption from intestines. Of the dietary triglycerides, about 25-30% is totally hydrolyzed by lipases into free fatty acids and glycerol, about 60-70% is partially hydrolyzed to fatty acids and monoglycerides, and about 5% is absorbed without any enzymic digestion.

1. **Digestion in stomach:** A lipase has been demonstrated in the human gastric contents. It has an optimum pH of about 5 and can hydrolyze the ester linkages in triglycerides of short-chain and medium-chain fatty acids. It has also been called *tributyrase* for its effectiveness in digesting the butter fat tributyrin containing three butyric acid (C_4) residues. But the source of this 'gastric' lipase of human stomach is unknown. Nor does it appear to digest significant amounts of

fat at the gastric pH of 1-2 and in the absence of the fat-emulsifying action of bile salts in stomach. 'Gastric' lipases of calves and suckling rats are secreted either from the pharyngeal region or from a serous gland at the back of tongue. Nevertheless, Olivecrona *et al* demonstrated some hydrolysis of milk fat by lipase in the stomach of suckling infants, rats and calves and even of adult man. Lipids of butter and egg-yolk occur in emulsions in food and may be significantly hydrolyzed by lipases in the stomach into diglycerides and fatty acids, needing no prior emulsification by bile salts. Lower gastric acidity in infants may also permit some gastric lipolysis of milk fat.

2. **Digestion in intestine:** In the small intestine, fats are digested by the coordinated actions of bile salts (glycocholate, taurocholate, etc.), pancreatic colipase and pancreatic and intestinal lipases. Phospholipids are digested by pancreatic and intestinal phospholipases and lysophospholipase. Cholesteryl esters are hydrolyzed by pancreatic cholesterol esterase.

(a) **Action of lipases and bile salts:** *Pancreatic lipase* is of prime importance in the hydrolysis of triglycerides. It is an esterase (pH optima about 8) and hydrolyzes primary ester linkages between fatty acids and primary alcohol groups of C^1 and C^3 (α - and α' -C) of glycerol. But it has very little action on the secondary ester linkage between a fatty acid and the secondary alcohol group of C^2 (β -C) of glycerol. So, pancreatic lipase digests a triglyceride first to 1,2-diglyceride (α,β -diacylglycerol) and then to 2-monoglyceride (β -monoacylglycerol), releasing a free fatty acid at each step (Fig. 8.13). 2-Monoglyceride may partly isomerize to 1-monoglyceride; but at the intestinal pH of 5.8-6.5 in which the lipase acts, 2-monoglyceride is fairly stable and has a very low rate of isomerization to 1-monoglyceride. Thus, in view of the poor hydrolytic action of the lipase on the

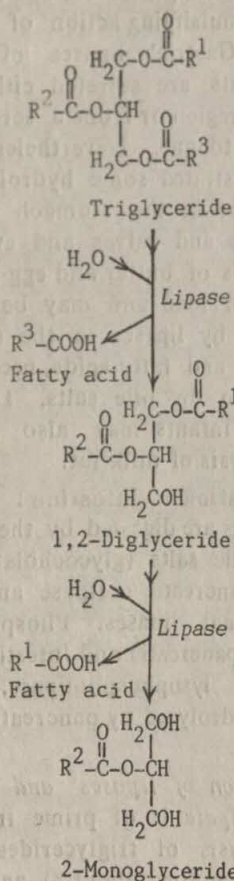


Fig. 8.13. Action of lipase.

secondary ester linkage in 2-monoglyceride, lipase action virtually ends at the monoglyceride stage. Lipase action on fats needs the presence of bile salts, pancreatic colipase and Ca²⁺ in the intestinal lumen.

Bile salts are the salts of *bile acids* such as taurocholic, glycocholic, taurochenodeoxycholic and glycochenodeoxycholic acids, synthesized and secreted by liver cells in bile. In the intestinal lumen, amphipathic molecules of bile salts concentrate on the surface of each fat droplet to form a surface monolayer. This lowers the surface tension of the fat droplet and consequently the interfacial tension

between the fat droplet and the surrounding aqueous medium. This causes tiny droplets to flow out from the surfaces of larger droplets and to form a stable fine emulsion in the aqueous medium (*emulsifying action* of bile salts). Emulsification of fat is further accentuated by the simultaneous presence of other amphipathic substances, particularly *phosphatidylcholines* and *monoglycerides*, in the intestinal lumen. By decimating large fat droplets into many more small ones, emulsification enhances the surface area of fat droplets and thereby increases lipase action on them, because water-soluble lipases can act only at the interface between the aqueous medium and insoluble fat droplets.

Colipase (MW ≈ 11 kdal) is a small protein secreted in the pancreatic juice. It binds to the surface of emulsified fat droplets and serves to displace locally the surface layer of bile salts and other detergents. Lipases cannot of themselves penetrate the surface monolayer of emulsifiers to reach the surface of fat droplet. But colipase action helps to anchor the lipase to the emulsifier-free loci on the surface of fat at the intestinal pH.

Calcium ions form insoluble calcium soaps with free fatty acids released by lipase action, and thereby prevent the free fatty acids from inhibiting further lipase action. Thus Ca²⁺ facilitates pancreatic lipase activity.

The importance of pancreatic lipase and bile in the digestion and absorption of fat is indicated by an abnormally high elimination of fat in feces of patients suffering from a dysfunction or destruction of exocrine pancreas, a failure of pancreatic juice to reach duodenum, an inherited deficiency of pancreatic lipase, obstructive jaundice or liver dysfunctions.

Intestinal lipase occurs mainly inside the intestinal epithelial cells. Its action is similar to that of pancreatic lipase, but is quantitatively far less important, being limited mainly to the *intracellular digestion* of small

amounts of undigested triglycerides and diglycerides absorbed into intestinal cells.

Both pancreatic and intestinal lipases have little action on 2-monoglycerides. After absorption into intestinal epithelial cells, 2-monoglycerides are used mainly for resynthesis of triglycerides. To a minor extent, they may also be isomerized to 1-monoglyceride (α -monoacylglycerol) by *monoacylglycerol isomerase*, and then hydrolyzed by intestinal lipase into fatty acid and glycerol—an instance of intracellular digestion.

A lipase of human colostrum and milk may reach the infant intestine without denaturation by gastric acidity. There, it may hydrolyze some milk fat in presence of bile salts.

(b) *Action of phospholipases*: Pancreatic juice contains inactive prophospholipase A_2 , which is activated into *phospholipase A_2* by trypsin in the duodenum. The enzyme hydrolyzes the secondary ester linkage between a fatty acid and the secondary alcohol group at C^2 of glycerol in the phospholipid molecule, cleaving the latter into a lysophospholipid (e.g., lysolecithin from lecithin) and a free fatty acid (Fig. 8.14). *Bile salts* help

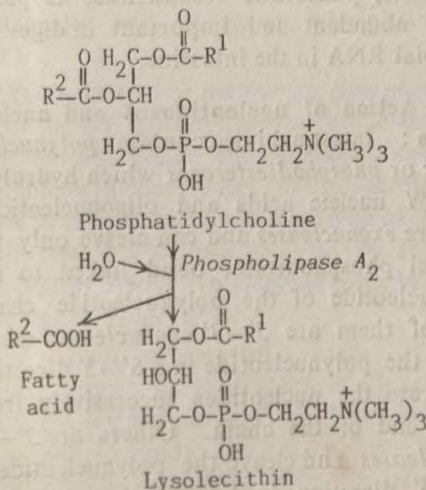


Fig. 8.14. Action of phospholipase A_2 .

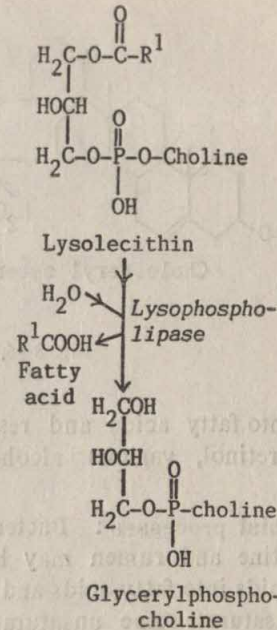


Fig. 8.15. Action of lysophospholipase.

in phospholipase action by emulsifying lipids into finer droplets with relatively more surface area; Ca^{2+} helps by forming insoluble calcium soaps with the released fatty acids. Intestinal mucosa contains a similar *intestinal phospholipase A* (lecithinase A) which also hydrolyzes phospholipids into lysophospholipids and fatty acids. Subsequently, *lysophospholipase* (lysolecithinase) of intestinal mucosa cells hydrolyzes the primary ester linkage between a fatty acid and the primary alcohol group of C^1 of glycerol in lysophospholipid molecule to cleave it to glycerylphosphocholine and a fatty acid (Fig. 8.15). The intestinal enzymes probably act mainly intracellularly on the absorbed phospholipids.

(c) *Action of cholesterol esterase*: Bile salts activate cholesterol esterase of pancreatic juice in the intestinal lumen. The enzyme hydrolyzes ester linkages in cholesteryl esters (Fig. 8.16), retinyl esters, lower fatty acyl esters and probably some 2-monoglycerides to

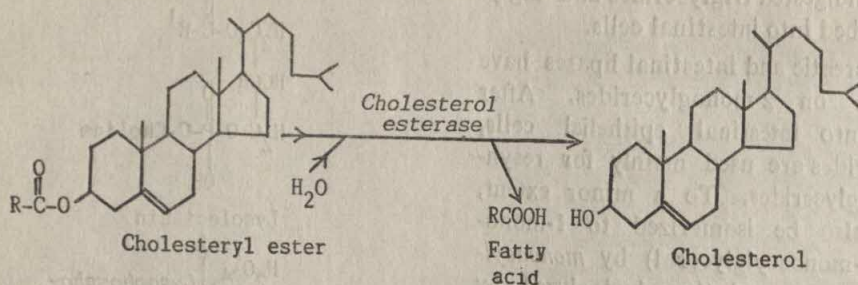


Fig. 8.16. Action of cholesterol esterase.

cleave them into fatty acids and respectively cholesterol, retinol, various alcohols and glycerol.

3. Microbial processes: Bacterial flora of large intestine and rumen may hydrolyze some plant lipids into fatty acids and glycerol, and may also saturate some unsaturated fatty acids of food by hydrogenation. Products may be absorbed by the host, particularly in case of ruminants. Bacterial hydrogenation of polyunsaturated fatty acids may reduce their availability to the host.

8.4 DIGESTION OF NUCLEIC ACIDS

Nucleoproteins are first digested by proteases to liberate free nucleic acids. Nucleic acids are then digested by pancreatic nucleases and intestinal nucleotidases and nucleosidases.

1. Action of nucleases: Pancreatic juice contains ribonuclease (RNase) and deoxyribonuclease (DNase). Both are *endonucleases* and hydrolyze phosphodiester linkages between nucleotides deep in the middle of the nucleic acid chain. At near-neutral intestinal pH and in presence of Mg^{2+} or Mn^{2+} , *pancreatic deoxyribonuclease I* hydrolyzes phosphodiester linkages between purine and pyrimidine nucleotides in DNA, liberating deoxyribonucleotides (e.g., deoxyadenosine 5'-P and deoxythymidine 5'-P) and oligodeoxyribonucleotides (e.g., deoxycytidylate-deoxycytidylate and deoxycytidylate-deoxyadenylate

dinucleotides). Bovine pancreatic deoxyribonuclease is an oligomeric protein and a *type a endonuclease*, cleaving only C-3'-P linkages in the DNA chain to produce a 5'-phosphoryl terminus in one product and a 3'-hydroxyl terminus in the other (Fig. 8.17). *Pancreatic ribonuclease*, a monomeric enzyme, is a *type b endonuclease* and hydrolyzes C-5'-P linkages in the single-stranded RNA to yield one product with a 3'-phosphoryl terminus and another with a 5'-hydroxyl terminus. RNase hydrolyzes RNA in this way to oligoribonucleotides. But it cannot hydrolyze RNA-DNA hybrid strands and double-stranded regions of RNA. In ruminants, pancreatic ribonuclease is particularly abundant and important in digesting microbial RNA in the intestine.

2. Action of nucleotidases and nucleosidases: Intestinal juice contains *polynucleotidases* or *phosphodiesterases* which hydrolyze low-MW nucleic acids and oligonucleotides. They are *exonucleases* and can cleave only the terminal phosphodiester bond linked to the last nucleotide of the polynucleotide chain. Some of them are 5'→3' *exonucleases* which cleave the polynucleotide in a 5'→3' direction to liberate the nucleotides successively from the 5' end of the chain. Others are 3'→5' *exonucleases* and cleave the polynucleotide in a 3'→5' direction to liberate the nucleotides successively from the 3' end of the chain.

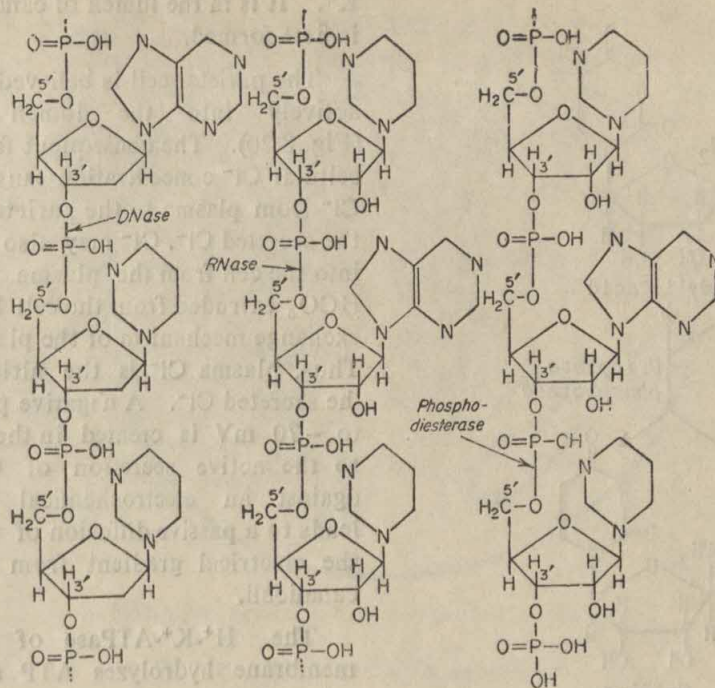


Fig. 8.17. Actions of nucleases and phosphodiesterase.

Intestinal juice contains *nucleotidases* or *nucleotide phosphatases*. They hydrolyze the phosphoester bond in specific purine or pyrimidine nucleotides to yield the respective nucleosides and inorganic phosphate (Fig. 8.18).

Nucleosidases or *nucleoside phosphorylases* occur in the microvillus membrane of intestinal mucosa cells. Purine nucleosidases and pyrimidine nucleosidases use inorganic phosphate to catalyze the phosphorolysis of specific nucleosides into the purine or pyrimidine base and either ribose 1-phosphate or deoxyribose 1-phosphate (Fig. 8.19).

3. Action of xanthine oxidase: This enzyme occurs in significant amounts in the intestinal epithelium and oxidizes hypoxanthine and xanthine, released from purine nucleosides by digestion, into uric acid. Its action consequently destroys much of the purines.

4. Microbial processes: Besides the intestinal xanthine oxidase, intestinal microorganisms also destroy much of the dietary purines and pyrimidines by putrefaction. So, very little of dietary purines and pyrimidines can be absorbed and utilized.

8.5 GASTRIC HCL

Gastric juice contains 0.3-0.5% HCl, the concentration rising with the rise in secretion rate. HCl is secreted by parietal (oxyntic) cells of gastric glands.

Functions of HCl: (a) HCl denatures many native proteins to *acid metaproteins* by cleaving the electrostatic and hydrogen bonds holding their tertiary structures. This enhances pepsin action on the unfolded peptide chain of denatured protein. (b) HCl lowers the gastric pH to 2 or below, causing activation of pepsinogen and prorennin to pepsin and rennin respectively. (c) It maintains an

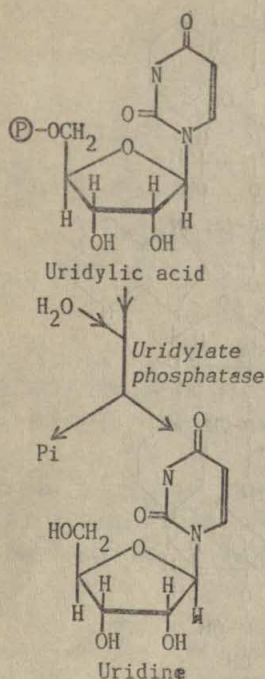


Fig. 8.18. Action of a nucleotide phosphatase.

optimum gastric pH (1-3) for protein digestion by pepsin. (d) It provides an optimum pH for the action of Castle's intrinsic factor, a gastric mucoprotein enzyme essential for vitamin B_{12} absorption. (e) HCl hydrolyzes some sucrose to glucose and fructose. (f) It has a strong bacteriolytic action and destroys many microorganisms entering with food and drink.

Origin of HCl : Parietal cells actively secrete an almost isotonic solution of HCl containing 150 mEq of H^+ per litre and having a pH lower than 0.87. The parietal cell has an extensive system of canaliculi, lined by its plasma membrane and opening into the adjoining lumen of the gastric gland. Many microvilli of the cell project into the canaliculi from their surface. The microvillus membrane contains an $\text{H}^+\text{-K}^+\text{-ATPase}$ which actively secretes H^+ into the canaliculi in exchange of

K^+ . It is in the lumen of canaliculi that HCl is first formed.

The parietal cell is believed to secrete Cl^- actively into the lumen of canaliculi (Fig. 8.20). The consequent fall in the intracellular Cl^- concentration causes a diffusion of Cl^- from plasma to the parietal cell to replace the secreted Cl^- . Cl^- may also be transferred into the cell from the plasma in exchange of HCO_3^- extruded from the cell by a $\text{Cl}^-/\text{HCO}_3^-$ exchange mechanism of the plasma membrane. Thus, plasma Cl^- is the ultimate source of the secreted Cl^- . A negative potential of -40 to -70 mV is created in the canaliculi due to the active secretion of Cl^- into them against an electrochemical gradient. This leads to a passive diffusion of some K^+ down the electrical gradient from the cell to the canaliculi.

The $\text{H}^+\text{-K}^+\text{-ATPase}$ of the microvillus membrane hydrolyzes ATP and utilizes the released energy in actively transporting H^+ of

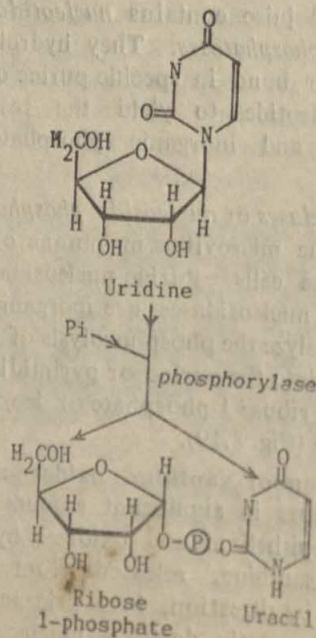
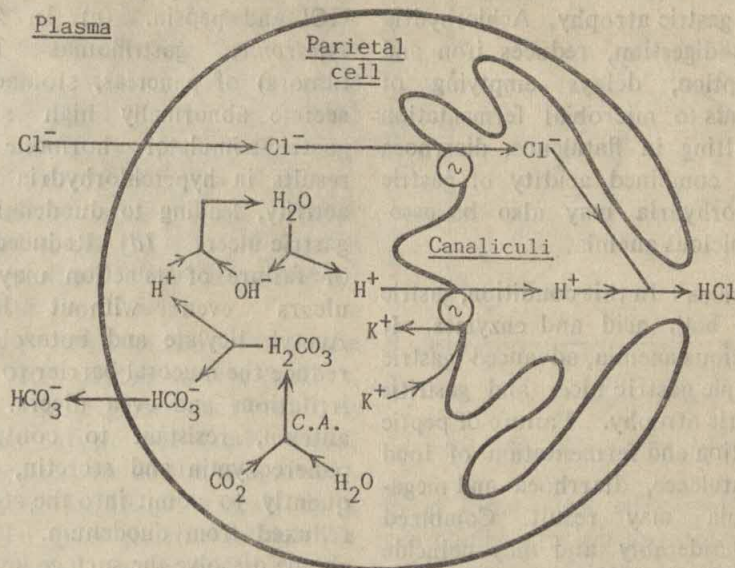


Fig. 8.19. Action of uridine phosphorylase.



(~) Active transport. C.A. = Carbonic anhydrase.

Fig. 8.20. Origin of HCl in parietal cell.

water across the membrane from the cytoplasm to the lumen of canaliculi and in exchange, recycles K^+ from the membrane to the cytoplasm. It has been demonstrated that the secretion of H^+ by isolated gastric glands or perfused gastric mucosa, requires the presence of K^+ in the perfusion medium and is depressed or abolished in K^+ -free medium. In the *in vivo* experiments using fundic pouches in dogs, substituted benzimidazoles inhibit the gastric secretion of H^+ by inhibiting the membrane H^+/K^+ -ATPase. Thiocyanate, on the other hand, inhibits the outward transport of H^+ by the membrane by altering the conformation of H^+/K^+ exchanger system, leaving the hydrolysis of ATP uninhibited.

Secretion of the H^+ of intracellular water into the canaliculi leaves behind the OH^- of water in the parietal cell. The consequent tendency of alkalinity in the cell is immediately neutralized by using CO_2 , supplied largely by blood and to a smaller extent by the metabolism of parietal cell itself.

This CO_2 uptake by parietal cells from blood explains the significantly lower CO_2 content of the gastric venous blood compared to the gastric arterial blood during gastric HCl secretion.

CO_2 is hydrated in parietal cells by *carbonic anhydrase* to form H_2CO_3 . The latter buffers the OH^- left behind by the secreted H^+ , producing H_2O and HCO_3^- . The gastric mucosa is rich in the Zn^{2+} -protein carbonic anhydrase. Inhibitors of this enzyme (e.g., acetazolamide) depress HCl secretion by as much as 80%. HCO_3^- produced by the buffering of OH^- passes out into the blood in exchange of Cl^- entering the cell from the blood. This raises the plasma HCO_3^- and the blood pH at the height of gastric secretion after a meal. The excess HCO_3^- is subsequently eliminated in the urine, raising the urinary pH (*postprandial alkaline tide*).

Achlorhydria: It is the absence of HCl in the gastric juice. This occurs in gastric carcinoma, atrophic gastritis, wasting diseases

of stomach and advanced chronic gastric ulcer leading to gastric atrophy. Achlorhydria prevents peptic digestion, reduces iron and vitamin absorption, delays emptying of stomach and leads to microbial fermentation in stomach resulting in flatulence, diarrhoea and rise in the combined acidity of gastric contents. Achlorhydria may also be associated with pernicious anemia.

Achylia gastrica : In this condition, gastric juice lacks in both acid and enzymes. It occurs in pernicious anemia, advanced gastric carcinoma, chronic gastric ulcer and gastritis leading to gastric atrophy. Failure of peptic digestion, retention and fermentation of food in stomach, flatulence, diarrhoea and megaloblastic anemia may result. Combined acidity rises considerably and may coincide with the total acidity of gastric contents.

Hyperchlorhydria : This is an abnormally high secretion of gastric HCl, raising the free acidity of gastric contents and producing chronic postprandial heartburns. Few cases of gastric carcinoma and some cases of gastric ulcer show hyperchlorhydria while many gastric ulcer patients have normal gastric acidity. But there is a high association between hyperchlorhydria and duodenal ulcer.

8.6 PEPTIC ULCERS

Peptic ulcers result from the excoriation of particularly upper duodenal, pre-pyloric or lower esophageal mucosa due to either increased gastric secretion or failure of protective mechanisms against the corrosive action of gastric juice. Duodenal ulcers are often associated with hyperchlorhydria, but gastric ulcer patients frequently have normal gastric acidity. Peptic ulcers may result from following factors. (a) Continued states of anxiety or tension may increase gastric secretion through prolonged vagal stimulation. (b) Reduced blood supply to stomach or duodenum may depress the secretion of mucin

which normally protects the mucosa against HCl and pepsin. (c) In *Zollinger-Ellison syndrome*, gastrinomas (gastrin-secreting tumors) of pancreas, stomach or duodenum secrete abnormally high amounts of the gastric-stimulator hormone gastrin. This results in hyperchlorhydria and high peptic activity, leading to duodenal and pre-pyloric gastric ulcers. (d) Reduced mucin secretion or failure of its action may lead to gastric ulcers even without hyperchlorhydria. Acetylsalicylate and butazolidine drugs may reduce the mucosal barrier to produce gastric irritations and even ulcers. (e) A pyloric antrum, resistant to contractile effects of pancreozymin and secretin, opens very frequently to admit into the stomach the chyme refluxed from duodenum. Bile salts of this chyme dissolve the surface layer of mucus on particularly the pre-pyloric mucosa, exposing it to corrosion by gastric juice. (f) Mechanical or chemical irritation of the gastric mucosa may cause traumatic loci susceptible to corrosive actions of gastric juice, particularly in the pyloric part.

8.7 FRACTIONAL GASTRIC ANALYSIS

For collecting gastric contents, a rubber tube with metal tip (Ryle's tube or Rehfsuss tube) is inserted through the mouth into the body of stomach of a human patient who has omitted breakfast or lunch according as the gastric contents are being collected in the morning or afternoon respectively. The stomach is then emptied of the residuum by aspiration through the stomach tube. The patient is next fed with a test meal of either oatmeal gruel or 5% ethanol or dry toasted breads and tea. Samples of gastric contents are then collected by aspiration through the tube every 15 minutes for 2½ hours. Each sample is strained through fine-mesh cheesecloth to separate food remains, mucus or blood. The strained sample is then subjected to following tests.

Free acidity: This is almost wholly due to free HCl which is practically ionized into H^+ and Cl^- . Free acidity normally ranges upto about 40 mEq litre⁻¹ (Fig. 8.21). It is estimated by titrating a gastric sample with 0.01 N NaOH, using as indicator either Topfer's reagent (changing from red to salmon-pink at a pH of about 3.3) or methyl orange (red $\rightarrow$ orange at about pH 3.6) or thymol blue (red $\rightarrow$ yellow at pH 2.8) or bromophenol blue (yellow $\rightarrow$ blue at pH 3.4).

Total acidity: This results from the presence of free HCl, protein hydrochlorides, acid phosphates and organic acids such as acetic, propionic, butyric and lactic acids. Total acidity may rise to 50-100 mEq litre⁻¹ after the first hour following the meal. It is estimated by titrating a gastric sample with 0.01 N NaOH, using as indicator either phenolphthalein (colorless $\rightarrow$ red at pH 8.5), phenol red (yellow $\rightarrow$ red at pH 8.2) or thymol blue (yellow $\rightarrow$ blue at a pH near 9).

Combined acidity: This is the acidity caused by weak organic acids, acid phosphates and protein hydrochlorides, and serves as a measure of the buffering capacity of the gastric juice. It is given by the difference between the total and free acidities of a gastric sample. Combined acidity rises in achlorhydria with food retention in stomach (e.g., in gastric carcinoma) because of the enhanced bacterial fermentation of the retained food into organic acids.

Peptic activity: To estimate pepsin activity, a gastric sample is incubated at 37°C with a known amount of I^{131} -bound albumin, the undigested albumin is finally precipitated with trichloroacetic acid, and the I^{131} in the remaining solution is then estimated by a scintillation counter. The results are compared with those obtained by incubating a known amount of pepsin, instead of the gastric sample, with I^{131} -albumin.

In another method, the gastric sample is incubated at 37°C with an acidic solution of

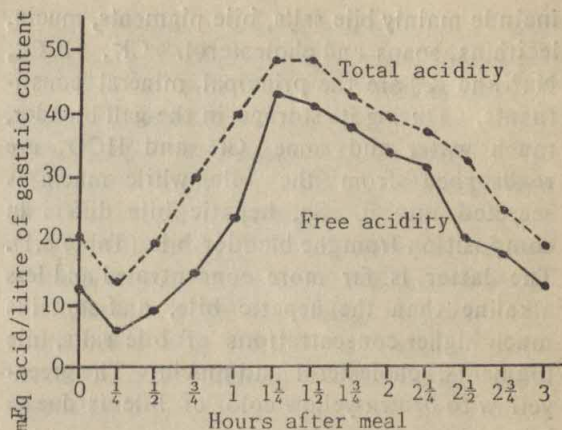


Fig. 8.21. Normal gastric acidity curves.

dried serum, the undigested serum proteins are finally precipitated with trichloroacetic acid, and the filtrate is treated with Folin-Ciocalteu phenol reagent. The reagent reacts with tyrosine, released by peptic digestion of serum proteins, to give a blue complex. The light transmittance of the latter is measured by a photometer.

Total chlorides: These normally vary from 40 to 155 mEq litre⁻¹. They include both HCl and neutral chlorides of Na^+ , K^+ and other metals. Ordinarily the concentrations of neutral chlorides and HCl vary inversely. To estimate total chlorides, a few drops of conc HNO_3 and a measured amount of 0.05 N $AgNO_3$ are added to the strained gastric sample to precipitate the chlorides as $AgCl$. The remaining unchanged $AgNO_3$ is titrated with 0.02 N NH_4CNS using ferric alum as indicator. The persistent salmon-red color of ferric thiocyanate indicates the endpoint. The amount of Cl^- in the sample is calculated from the amount of $AgNO_3$ used up in precipitating it.

8.8 BILE CONSTITUENTS

Bile is secreted by the liver and stored in the gall bladder before its expulsion into the duodenum. The organic constituents of bile

include mainly bile salts, bile pigments, mucin, lecithins, soaps and cholesterol. Cl^- , HCO_3^- , Na^+ and K^+ are the principal mineral constituents. During its storage in the gall bladder, much water and some Cl^- and HCO_3^- are reabsorbed from the bile while mucin is secreted into it. So, hepatic bile differs in composition from the bladder bile (Table 8.1). The latter is far more concentrated and less alkaline than the hepatic bile, and contains much higher concentrations of bile salts, bile pigments, cholesterol and mucin. The green-yellow to brown-yellow color of bile is due to bile pigments.

Bile salts: These are sodium and potassium salts of bile acids which are cholic acid conjugates such as glycocholic, taurocholic, glycochenodeoxycholic and taurochenodeoxycholic acids. In the human bile, glycocholate and taurocholate are the principal bile acids, the taurine conjugates amounting to only about $\frac{1}{3}$ rd of the amount of glycine conjugates. Bile acids are synthesized by the hepatic cells from cholesterol at the rate of upto 500 mg day^{-1} and are considered the principal catabolites of cholesterol. (For the synthesis of bile acids, see cholesterol metabolism in Chapter 11). About 90% of the bile salts, secreted in the duodenum, may be reabsorbed from ileum and carried by blood to the liver for resecretion into bile (*enterohepatic biliary circulation*). Remaining 10% of the bile salts escapes reabsorption to be excreted in the feces after being partly putrefacted to lithocholic and deoxycholic acids. Fecal bile salts amount to $200\text{--}500 \text{ mg day}^{-1}$.

Functions: Bile salts serve mainly in digestion and absorption.

(a) *Digestion of lipids:* (i) Amphipathic molecules of bile salts concentrate on the surface of each fat droplet in the intestine to form a monolayer over it. This lowers the surface tension of the fat droplet and consequently the interfacial tension between the fat droplet and the surrounding aqueous medium.

So, tiny droplets flow out from the surface of large fat droplets to form a stable emulsion in the aqueous medium (*emulsifying action*). By dividing large fat droplets into many more small ones, emulsification increases the surface area of fat droplets and thereby enhances lipase action on them, because water-soluble lipases can act only at the water-lipid interface. (ii) Bile salts activate pancreatic cholesterol esterase in the intestinal lumen.

(b) *Absorption of lipids:* (i) The emulsifying action of bile salts on fat is further accentuated in presence of other amphipathic substances like lecithins and products of fat digestion like monoglycerides and soaps. As a result, a small amount of undigested triglycerides is so finely emulsified that it is absorbed without any hydrolysis. (ii) Because of the amphipathic nature of their molecules, bile salts, soaps and phosphoglycerides form molecular aggregates called *micelles* in the intestine (vide 2.11). The polar groups of these molecules form the water-adjointing outer surface of the micelle while their non-polar groups are placed deep inside the non-aqueous interior of the micelle. Products of lipid digestion such as higher fatty acids, monoglycerides, etc., get incorporated in the nonaqueous interior of the micelle. Being covered by the superficial layer of polar groups of bile salts and other amphipathic substances, these *mixed micelles* remain finely dispersed in the aqueous intestinal contents (*hydrotropic action* of bile salts). Lipids and sterols are easily absorbed in the intestine from such mixed micelles. In obstructive jaundice, bile salts fail to reach the intestine while in liver dysfunctions, they fail to be secreted. In these conditions, micelle formation is depressed in the intestine with consequent elimination of large amounts of unabsorbed fat in the feces (*steatorrhoea*).

(c) *Absorption of vitamins:* Bile salts help in the absorption of carotenes and vitamins D, E and K by forming mixed micelles

into which these materials get incorporated. In obstructive jaundice and liver dysfunctions, bile salts fail to be secreted or to reach the intestine. This reduces the absorption of these vitamins leading to their deficiency symptoms.

(d) *Digestion of proteins*: Bile salts liberate enteropeptidase into the intestinal lumen from the microvillus membrane of duodenal mucosa cells. Besides, they may probably preserve protease activities of pancreatic enzymes by counteracting against the inhibitory effects of fatty acids.

(e) *Stimulation of bile secretion*: As bile salts, reabsorbed from the intestine, are recycled through the liver, they enhance the bile flow into the intestine (*cholagogue effect*) in two ways. Glycocholates and taurocholates stimulate hepatic cells to secrete both bile salts and water (*choleretic action*) while deoxycholate conjugates stimulate only water secretion into bile (*hydrocholeretic action*).

(f) *Intestinal peristalsis*: Bile salts stimulate intestinal peristalsis, thereby minimize the stay of food in the intestine and consequently restrict bacterial putrefaction.

(g) *Regulation of cholesterol synthesis*: Bile salts, reabsorbed from the intestine, exert an inhibitory control on the hepatic synthesis of cholesterol by a feed-back inhibition of HMG-CoA reductase, the rate-limiting enzyme of the pathway.

(h) *Excretion of cholesterol*: Bile salts help in the excretion of cholesterol in bile by forming mixed micelles in it with mainly lecithins and cholesterol. Solubility of cholesterol depends on the relative proportions of these substances in the micelles of bile. In fact, the ratio of concentrations of bile salts, lecithins and cholesterol is about 16 : 3 : 1 in the bladder bile and falls within the optimal range for keeping both cholesterol and lecithins in solution.

Cholesterol: Biliary cholesterol comes partly from the cholesterol removed by hepatic cells from blood and partly from the cholesterol synthesized in the liver. Bile is the main channel for the excretion of cholesterol. Cholesterol is kept in solution in aqueous bile by being incorporated into mixed micelles with the help of bile salts and lecithins. Some of the biliary cholesterol is reabsorbed from the intestine. The remaining cholesterol is eliminated with the feces, largely after bacterial putrefaction to cholestanol and coprostanol.

Bile pigments: These consist mainly of bilirubin and biliverdin formed by the degradation of the iron-free porphyrin of hemoglobin by macrophages of spleen, liver and bone marrow. (For details, see Chapter 9). Bilirubin, released by macrophages, is removed by hepatic cells from blood, conjugated with glucuronic acid to form bilirubin glucuronides and excreted in bile. Bile pigments are eliminated with the feces, largely after bacterial putrefaction to stercobilinogen.

8.9 JAUNDICE

Jaundice is an abnormal rise in the serum bilirubin, causing yellow coloration of skin, mucous membranes and sclera and a rise in urinary bilirubin.

Hepatic jaundice: In liver dysfunctions such as cirrhosis or necrosis of liver and infective hepatitis, liver cells fail to remove unconjugated bilirubin from blood and to conjugate and excrete it in bile. This increases unconjugated bilirubin in blood and urine.

Obstructive jaundice: Obstructions in bile passages due to tumors or gall stones may lead to regurgitation of bile, containing bilirubin glucuronides, into the blood. This increases conjugated bilirubin in both blood and urine.

Hemolytic jaundice: Excessive destruction of erythrocytes produces large amounts

of bilirubin in hemolytic anemias, malaria and incompatible blood transfusions. Liver cells fail to remove all the unconjugated bilirubin from blood for excretion in bile. Mainly unconjugated bilirubin rises in blood and urine.

Crigler-Najjar syndrome: Unconjugated bilirubin rises in blood and urine in these diseases due to an inborn deficiency of either bilirubin-UDP glucuronyl transferase or the hepatic enzyme converting bilirubin monoglucuronide to bilirubin diglucuronide.

8.10 ABSORPTION OF CARBOHYDRATES

Hexoses and small amounts of pentoses and disaccharides are absorbed from the small intestine.

Absorption of pentoses: Pentoses are absorbed from the small intestine by the physical process of *diffusion*. So, the rate of absorption of a pentose is slow and depends on its concentration gradient, i.e., the difference in its concentration in the intestinal contents and the blood. Absorption continues as long as the concentration of the pentose is higher in the intestinal contents than in the blood and stops as soon as the concentration becomes almost identical in the intestinal lumen and blood. So, the total amount of a pentose present in the intestinal contents cannot be absorbed by diffusion. Because diffusion neither depends upon cellular activity nor needs energy expenditure, pentose absorption is unaffected by hypoxia, cold and metabolic inhibitors.

Absorption of glucose and galactose: D-Glucose and D-galactose are absorbed mainly from the duodenum and jejunum by a *carrier-mediated active transport*, depending upon the simultaneous active absorption of Na^+ . Besides, small amounts of these hexoses may also be absorbed at much slower rates from small intestine as well as colon by diffusion as long as there exist concentration gradients of these sugars between the intestinal contents and the blood.

The process of active absorption is far more rapid than diffusion, necessitates *cellular work*, involves *energy expenditure*, takes place *unidirectionally* (always from the intestinal lumen to the blood and not in the reverse direction), absorbs only *specific substrates* (e.g., D-glucose and D-galactose), continues *against concentration and electrical gradients* and may consequently absorb the substrates completely from the intestinal contents. Active absorption involves integral membrane proteins acting as *carriers* for specific substrates. The carrier protein binds with its substrate on a specific surface of the cell membrane, transfers the substrate unidirectionally across the membrane and releases it from the other surface of the membrane. The rate of active transport cannot exceed a maximum level even with a very high substrate concentration, indicating that the carrier gets *saturated* with the substrate at a high concentration of the latter. Substances structurally similar to the substrate may *competitively inhibit* this active transport. Some evidences for active absorption of D-glucose and D-galactose are mentioned below. (a) D-Glucose and D-galactose are absorbed at very high rates, higher than what is expected from their diffusion coefficients and more rapid than the absorption rates of more diffusible pentoses. (b) Absorption of D-glucose and D-galactose continues against their respective concentration gradients—intestinal absorption of glucose continues even when the mesenteric blood glucose level far exceeds the glucose concentration in the intestinal lumen. (c) If glucose solutions of two different concentrations are separated by a piece of excised intestinal mucosa, glucose continues to pass from the mucosal to the serosal side of the mucosa even when the glucose concentration is higher on the serosal side. (d) During the absorption of glucose or galactose, the mucosal surface of the intestinal epithelium becomes more electronegative than its serosal surface indicating active cellular work and

transport of cations (Na^+) from the mucosal to the serosal side. (e) The rate of glucose absorption is considerably reduced and the electronegativity of the mucosal surface of intestinal epithelium is simultaneously abolished on the application of cold, metabolic inhibitors, excess of K^+ or Li^+ , or cardiac glycosides like ouabain which depresses active Na^+ absorption; the slow rate of glucose absorption under such conditions corresponds to the diffusion rate of glucose. All these findings indicate that these two hexoses are mainly absorbed by an active, carrier-mediated, Na^+ -dependent process. It is now believed that D-glucose is absorbed simultaneously with the active absorption of Na^+ by a process called *symport*. (For details, see page 126).

D-Galactose is more rapidly absorbed than D-glucose. Both the hexoses share a common carrier, because increase in the intestinal concentration of one lowers the rate of absorption of the other. The carrier protein for the active absorption of hexoses seems to possess specificity for only D-sugars having 6 or more carbons, a pyran ring, a methyl or substituted methyl group on C^3 and a free $\text{C}^2\text{-OH}$ group in the same spatial configuration as in D-glucose. During active absorption, the sugar undergoes mutarotation in the intestinal epithelium with the help of a mutarotase.

Absorption of fructose and mannose: Fructose and mannose are absorbed from the small intestine by *facilitated diffusion*. Like simple diffusion, it is also a physical process. It necessitates no energy expenditure, is unaffected by metabolic inhibitors, takes place only down a concentration gradient of the substrate and may occur both ways across a membrane, its direction depending on the direction of the concentration gradient. But it is far more rapid than simple diffusion, though much slower than active transport. Like the latter, facilitated diffusion is mediated by specific

integral membrane proteins acting as *carriers* to transfer specific substrates across the cell membrane. Thus, the carrier-mediated absorption of fructose or mannose is much faster than the diffusion of pentoses, but much slower than the active absorptions of glucose and galactose. Fructose or mannose continues to be absorbed even after application of metabolic inhibitors on the intestinal mucosa. Their absorptions depend on their respective concentration gradients between the intestinal contents and the blood, and stop when the concentrations become identical at the two sites. So, unlike glucose and galactose, fructose and mannose cannot be totally absorbed from the intestine.

Absorption of disaccharides: Intestinal disaccharidases, occurring in the microvillus membrane of intestinal mucosa cells, hydrolyze the disaccharides while transporting them across the membrane into the intestinal cell. In this way, lactose is absorbed from duodenum and jejunum, maltose from jejunum and proximal ileum, and sucrose from ileum and distal jejunum. Hexoses, produced by their intracellular digestion, are released from the intestinal cells into the blood.

After absorption, monosaccharides enter mainly the portal blood.

8.11 ABSORPTION OF PROTEINS

Amino acids are absorbed mainly from ileum and distal jejunum. Small oligopeptides are absorbed mainly from duodenum and proximal jejunum.

L-Amino acids and L-peptides are absorbed from the small intestine mainly by a *carrier-mediated active transport*, dependent upon the simultaneous active absorption of Na^+ . Evidences are as follows. (a) Rate of absorption of an L-amino acid is considerably higher than that of its D isomer. (b) Absorption rates differ for different L-amino acids. Basic and dicarboxylic amino acids are

generally absorbed more slowly than neutral amino acids. (c) Absorption rates of L-amino acids are independent of their diffusion coefficients and concentration gradients. (d) If two solutions of an L-amino acid are separated by an excised intestinal mucosa, the amino acid continues to pass from the mucosal to the serosal side of the mucosa even when its concentration is higher on the serosal side (e) Cold, hypoxia, metabolic inhibitors such as dinitrophenol and cyanide and excess of Li^+ or K^+ may depress L-amino acid absorption by inhibiting active transport. (f) High concentrations of one L-amino acid sometimes reduce the absorption of some other L-amino acids. This indicates that several L-amino acids may share a common carrier protein and compete with each other for occupying it. Unlike L-amino acids, D-amino acids are absorbed by diffusion, obeying their concentration gradients.

Different classes of L-amino acids, viz., diamino acids, dicarboxylic amino acids, small neutral amino acids and large neutral amino acids are believed to be absorbed by different carrier molecules present in the microvillus membrane of intestinal mucosa cells. Pyridoxal phosphate helps in the active absorption of amino acids. It has been postulated that L-amino acids are absorbed simultaneously with the active absorption of Na^+ by a process called *symport* (vide page 126).

L-Oligopeptides are hydrolyzed into amino acids by peptidases of the microvillus membrane of intestinal cells during their active absorption.

In new-born infants, globulins of colostrum are absorbed in unchanged forms from the intestine in preference to albumins. Specific globulins (e.g., immunoglobulin G) of the colostrum get concentrated on some depressed areas of the plasma membrane by binding with specific receptor molecules on the membrane. Such depressed membrane areas, bristling on their cytoplasmic surface

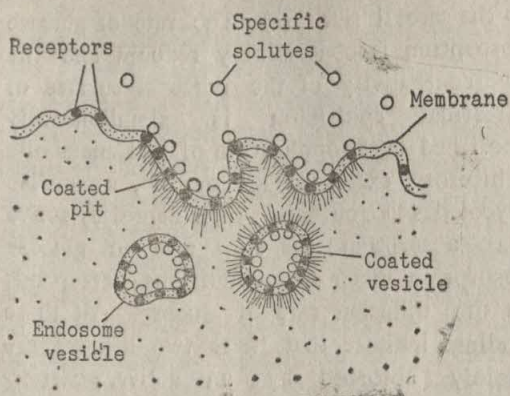


Fig. 8.20. Adsorptive pinocytosis.

with filamentous networks of a protein *clathrin*, are called *coated pits* (Fig. 8.20). The coated pit deepens into the cytoplasm and gets pinched off from the membrane as a free-floating *coated vesicle* with the receptor-bound proteins on the luminal surface of its membrane. The vesicle soon loses its clathrin coat to change into an uncoated *endosome vesicle* which migrates through the cytoplasm to the basal plasma membrane of the cell and discharges its contents into the blood. This process is called *adsorptive pinocytosis*. This capacity of absorbing unhydrolyzed proteins becomes much limited in a couple of weeks after birth.

8.12 ABSORPTION OF LIPIDS

About 95% of the dietary triglycerides are partially or totally hydrolyzed by lipases and their digested products are absorbed as free fatty acids, glycerol, monoglycerides and diglycerides. Of the products of triglyceride hydrolysis, lower fatty acids and glycerol are fairly soluble in water and are easily absorbed from jejunum and proximal ileum. They are then carried away by the portal blood. Absorption of lower fatty acids is an active and aerobic process, independent of concentration gradients.

Higher fatty acids, monoglycerides and diglycerides are insoluble in water and are absorbed with the help of bile salts. At the alkaline intestinal pH maintained by pancreatic and biliary HCO_3^- ions, bile salts, soaps and lecithins cooperate to collect the molecules of higher fatty acids, monoglycerides, diglycerides, cholesterol, fat-soluble vitamins, etc., into water-soluble molecular aggregates called *mixed micelles* (*hydrotropic action* of bile salts). From these mixed micelles, the digestion products of lipids are absorbed in duodenum, jejunum and proximal ileum. Inside the intestinal epithelial cells, most of the absorbed 2-monoglycerides (β -monoacylglycerols), higher fatty acids and diglycerides are directly used in synthesizing triglycerides. Some of the higher fatty acids are also used for phospholipid synthesis in these cells. α -Glycerophosphate, used in synthesizing triglycerides and phospholipids in the intestinal cells, comes either from glycolysis or from the intracellular hydrolysis of absorbed 1-monoglycerides (α -monoacylglycerols) by intestinal lipase. The synthesized triglycerides, along with some cholesterol, are aggregated into droplets, each of which is covered superficially by a hydrophilic layer of phospholipids and proteins to form a *chylomicron* droplet (diam. 0.5-3 μm). Chylomicrons pass out through the basal and lateral plasma membrane of intestinal cells and are carried away by lymph. Chylomicrons contain about 88% triglyceride, 4% phospholipid, 6% cholesterol and 2% protein, and have a density lower than 0.96.

About 5% of the food fat may be absorbed in highly emulsified form without any hydrolysis. Bile salts, monoglycerides and soaps have to act simultaneously on triglycerides to emulsify them into tiny droplets (diam. 0.5 μm or less) before they can be so absorbed from the small intestine.

Lysophospholipids and fatty acids, produced by the digestion of phospholipids,

are also incorporated into mixed micelles and absorbed from these micelles in the small intestine. About 20% of dietary phospholipids may be absorbed without digestion. Some of the phospholipids enter the chylomicrons and are carried in them to the lymph. Some phospholipids and free fatty acids are incorporated into *very low density lipoproteins* (VLDL) in the intestinal cells and released into lymph. Some phospholipids may also enter the portal blood.

Cholesterol is absorbed from mixed micelles in jejunum and ileum. It is re-esterified into cholesteryl esters in the intestinal cell. Both free and esterified cholesterol get incorporated in chylomicrons and are carried away in lymph.

Because of *active absorption* independent of concentration gradient, almost all the food fats are absorbed ordinarily. But failure of entry of bile or pancreatic juice into the duodenum, or intestinal lesions (e.g., in sprue) may reduce fat absorption and cause elimination of much fat in feces (*steatorrhoea*). Cold, hypoxia and metabolic inhibitors also depress fat absorption from isolated intestinal loops.

8.13 PUTREFACTION

Putrefaction is the process of microbial degradation of various food remnants and excreted materials in the large intestine. Putrefactions of proteins and amino acids produce toxic amines (*ptomaines*) such as agmatine and putrescine from arginine, histamine from histidine, cadaverine from lysine, indole ethanolamine from tryptophan, and tyramine from tyrosine. Aromatic compounds such as phenols, skatoles, indoles, p-cresols and benzoic acid, methyl and ethyl mercaptans, some free amino acids and considerable volumes of methane, H_2S , NH_3 , H_2 and CO_2 are also produced by protein putrefaction. Cholesterol is putrefacted to cholestanol and coprostanol, bile acids to deoxycholic and

lithocholic acids, and choline to neurine. Bilirubin glucuronide is hydrolyzed to free bilirubin and then reduced stepwise to stercobilinogen. Most of the products of putrefaction are eliminated in the feces.

8.14 DETOXICATION

Detoxication is the process of changing toxic substances and wasteproducts by mainly hepatic enzymes into less toxic and more soluble products for easy elimination in urine and bile. It involves the same enzymes as operate in normal metabolic pathways on substrates analogous to the toxic substances. A substance may be detoxicated by one or more of the following processes.

1. *Hydrolysis*: Procaine, for example, is hydrolyzed into diethylaminoethanol and para-aminobenzoate, and aspirin into acetate and salicylate.

2. *Oxidation*: Alcohols and aldehydes are oxidized to acids and even to CO_2 and H_2O , amino acids to NH_3 and keto acids, and S-compounds into sulfones and sulfoxides; e.g., oxidation of *n*-propanol to CO_2 and H_2O , and oxidation of benzaldehyde to benzoic acid.

3. *Reduction*: Aldehydes and ketones may be reduced to alcohols, aliphatic and aromatic disulfides (e.g., dibenzyl disulfide) to sulfhydryl derivatives like benzylthiol, and unsaturated compounds to respective saturated products.

4. *Conjugation*: Toxic substances or their detoxicated products from previous processes are frequently conjugated with some normal metabolic products before excretion in urine or bile. For example, (i) benzoic and indoleacetic acids are conjugated with glycine to produce hippuric and indoleacetic acids respectively. (ii) Glucuronyl transferases transfer glucuronic acid from UDP-glucuronic acid to bilirubin, steroids, amines,

alcohols and SH-compounds to form glucuronides such as bilirubin glucuronide and aldosterone glucuronide. (iii) Methyltransferases transfer the labile methyl group from *S*-adenosylmethionine to many compounds like nicotinamide, quinoline, thyroxine and estrogens. (iv) Sulfate transferases transfer SO_4^{2-} from adenosine 3'-phosphate-5'-phosphosulfate to the alcoholic OH of phenol, cresol, skatoles, indoles and steroids, and to the NH_2 of aliphatic and aromatic amines to form various *etheral sulfates*. (v) Cysteine is transferred from glutathione to aromatic hydrocarbons and halogenated derivatives of hydrocarbons and nitrobenzenes to produce their S-cysteine derivatives; e.g., p-bromophenylcysteine from bromobenzene. (vi) Acetylases transfer acetyl group from acetyl-CoA to the NH_2 groups of aromatic amines and S-cysteine derivatives; e.g., acetylation of p-bromophenylcysteine to p-bromophenyl mercapturic acid.

8.15 FECES

Normally about 150-200 g of feces are voided by an adult man in 24 hours. Feces are normally slightly alkaline or neutral, soft and formed, brown in color (due to stercobilin) and disagreeable in odour (owing to mercaptans, indole, skatole, H_2S and phenols).

Feces ordinarily contain 60-75% water and 25-40% solids. Unabsorbed food remnants form about 10% of feces and consist of mainly cellulose fibres, small amounts of scleroproteins, plant sterols, phytates, vegetable protein fibres and unabsorbed minerals, and very little fat.

About 10% of fecal matter consists of microorganisms such as *Escherichia coli*, *Enterobacter aerogenes*, *Proteus vulgaris* and *Clostridia welchii*. Fecal bacteria contribute most of the fecal proteins, nucleic acid and lipids.

Secreted or excreted materials constitute about 7-15% of feces. They include cholesterol, its putrefaction products (cholestanol and coprostanol), stercobilinogen, mucin, minerals and small amounts of bile salts and enzymes.

Occult blood in feces: In many gastrointestinal lesions such as gastritis, trauma, bleedings and malignancies, feces may contain altered blood, not detected visually. An aqueous suspension of feces is boiled to destroy peroxidases. It is then treated with 3% benzidine in glacial acetic acid and 10% H_2O_2 . If occult blood is present, a blue-green color appears due to the oxidation of benzidine with H_2O_2 , catalyzed by iron-containing derivatives of hemoglobin.

8.16 INBORN ERRORS OF DIGESTION AND ABSORPTION

Lactose intolerance and lactosuria: An inborn deficiency of lactase in the intestinal mucosa produces a failure of lactose digestion and absorption and a consequent intolerance of dietary lactose (*inherited lactase deficiency*). A gradual decline in lactase activity in the adult intestine with age may also produce similar lactose intolerance (*primary low-lactase activity*). Lactose intolerance may also result from intestinal lesions in sprue, gastroenteritis, kwashiorkor and colitis (*secondary low-lactase activity*). In all such cases of lactase deficiency, intestinal fermentation of undigested lactose produces large volumes of gases. This leads to bloating, flatulence, intestinal cramps and abdominal pain. The osmotic effect of unabsorbed lactose draws and retains large volumes of water in the intestinal contents, producing diarrhoea. Some of the undigested lactose may get absorbed, causing lactose to appear in the urine (lactosuria).

Sucrose-isomaltose intolerance: A rare inborn deficiency of both sucrase and isomaltase in the intestinal mucosa causes a failure

of sucrose and isomaltose digestion, leading to the intestinal fermentation of undigested sucrose and isomaltose, flatulence, diarrhoea and intestinal cramps. Absorption of undigested sucrose may lead to sucrosuria.

Pancreatic lipase deficiency: A congenital failure of pancreatic lipase secretion leads to a failure of fat digestion and absorption in the intestine. Unabsorbed fat is eliminated in the feces (*steatorrhoea*).

Glucose-carrier deficiency: An inborn deficiency of the glucose-carrier protein in the intestinal mucosa causes a failure of active absorption of glucose as well as galactose from the intestine. Fermentation of the unabsorbed sugars produces large volumes of gases in the intestine, leading to flatulence and abdominal pain. Osmotic effects of unabsorbed sugars enhances the volume of fluid in the intestinal contents, leading to diarrhoea. Growth rate is also reduced.

Pernicious anemia: This results from a failure of absorption of vitamin B_{12} due to an inborn deficiency of Castle's intrinsic factor in the gastric juice. (Vide 7.9).

8.17 STEATORRHOEA

Steatorrhoea is an abnormal rise of fat in the feces owing to a failure in digestion and absorption of fat. Large amounts of unabsorbed fat make the feces bulky, greasy, frothy, clay-colored and formless. A simultaneous failure of absorption of fat-soluble vitamins may sometimes produce symptoms like low plasma prothrombin and prolonged clotting time. Ca^{2+} absorption may also be reduced due to the formation of insoluble calcium soaps of unabsorbed fatty acids.

The fecal fat in steatorrhoea consists mainly of unhydrolyzed triglycerides (*unsplit fat*) if steatorrhoea has been caused by a failure of fat digestion due to reduced activity of pancreatic lipase as happens in (i) a failure of pancreatic juice to enter the duodenum,

(ii) a destruction of pancreatic exocrine tissue by trauma or diseases or (iii) an inborn deficiency of pancreatic lipase. The fecal fat consists mainly of fat hydrolysis products (*split fat*) like fatty acids and soaps if steatorrhoea has been caused by a failure of absorption of fat; such may happen in case of (i) intestinal lesions (e.g., in sprue, coeliac disease, chronic dysenteries and intestinal tuberculosis), (ii) a failure of bile secretion (e.g., in liver dysfunction) or (iii) a failure

of entry of bile into duodenum (e.g., in obstructive jaundice). Failure of action of bile on fat produces steatorrhoea due to a depression of micelle formation and a consequent failure of fat absorption. Gastrinomas or gastrin-secreting tumors may also produce steatorrhoea by causing excessive secretion of gastric HCl, because HCl lowers the intestinal pH to prevent micelle formation, precipitates some bile salts and reduces the action of pancreatic lipase on fat.

9. EXTRACELLULAR FLUIDS

Extracellular fluids (ECF) form about 45% of the total body water and occupy a total volume of about 14-18 litres in man. They include blood plasma, interstitial fluid and lymph, transcellular fluids like aqueous humor and cerebrospinal fluid, and extracellular fluids in dense connective tissues.

9.1 COMPOSITION OF BLOOD

Blood consists of *formed elements*, viz., erythrocytes, leucocytes and platelets, suspended in a yellowish solution called *plasma*. Blood has a considerable viscosity (3.6-5.4) and an average specific gravity of 1.060.

On centrifuging a sample of oxalated blood, erythrocytes collect at the bottom of the centrifuge tube forming a thick red layer, leucocytes form a thin 'buffy' layer on the upper surface of the erythrocyte layer and the plasma separates as the straw-colored supernatant fluid. The cells are then found to constitute 40-45% of the blood volume; this is the *hematocrit value* or *packed cell volume*. The remaining 55-60% of blood is the plasma. Adult human body contains about 4-5.5 litres of blood.

$$\text{Blood volume (ml)} = \frac{\text{Plasma volume (ml)} \times 100}{100 - \text{Packed cell volume}}$$

Blood, kept without adding anticoagulants such as oxalate or fluoride, coagulates in a few minutes and a red coagulated mass (*blood clot*) separates from the straw-colored supernatant fluid (*serum*). Serum, unlike plasma, is devoid of fibrinogen and cannot coagulate. The blood clot is a mass of blood cells caught in the meshwork of insoluble fibrin, formed from

the soluble protein fibrinogen during coagulation.

Plasma is a straw-colored, slightly alkaline (pH 7.4), slightly viscous (1.7-2) fluid with a specific gravity of about 1.026. It contains crystalloids and colloids in solution (Table 9.1); they exert an osmotic pressure of about 5453 mm Hg. Gases like O_2 , CO_2 and N_2 as well as hormones are also present in the blood.

9.2 FUNCTIONS OF BLOOD

1. **Transport:** Many substances are transported by blood. Blood helps in *respiration* by carrying oxygen from the lungs to the tissues and carbon di-oxide from the tissues to the lungs. It transports metabolic waste-products like urea, uric acid and ammonia, many toxins and drugs to the excretory organs and thus helps in *excretion*. It carries nutrients like glucose, fatty acids and amino acids to the tissues for their *nourishment* and also to liver and adipose tissues for *storage*. It *transports hormones* to their sites of action. It helps in the correlation of different steps of metabolic reactions by *transporting metabolites*.

2. **Body defence:** Phagocytic leucocytes phagocytize microorganisms and cellular debris. Lymphocytes synthesize antibodies of plasma, which help to inactivate toxins and antigens. Some plasma proteins, called *complements*, also help in body defence.

3. **Homeostasis:** Homeostasis is the maintenance of the internal environment of the body within narrow and definite limits with the help of physiological processes.

TABLE 9.1. *Some constituents of blood, plasma or serum. Values expressed per 100 ml of the fluid. IU : international units.*

Constituents	Normal ranges	Constituents	Normal ranges
Water (blood)	77-81 ml	Ketone bodies (plasma)	0.5-2.5 mg
Water (plasma)	91-92 ml	Cholesterol (plasma)	40-70 mg
Total solids (blood)	19-23 g	Cholesterol esters (plasma)	85-180 mg
Total solids (plasma)	8-9 g	Vitamin A (plasma)	20-60 μ g
Total proteins (plasma)	6-7.5 g	Carotenoids (serum)	25-300 μ g
Albumin (serum)	2.8-4.5 g	Vitamin C (plasma)	0.5-1.5 mg
Globulins (serum)	1.5-2.5 g	Thiamin (blood)	6-9 μ g
Fibrinogen (plasma)	200-500 mg	Riboflavin (blood)	15-22 μ g
Prealbumin (plasma)	30 mg	Niacin (blood)	0.5-0.7 mg
Hemoglobin (blood)	12-16 g	Biotin (blood)	0.05-0.15 μ g
Total NPN (blood)	20-35 mg	Pantothenate (blood)	15-30 μ g
Urea (blood)	17-30 mg	α -Tocopherol (serum)	0.5-1 mg
Ammonia (blood)	40-70 μ g	Bilirubin (plasma)	0.3-1 mg
Amino acids (blood)	31-47 mg	Amylase (serum)	60-180
Uric acid (plasma)	3-6 mg		Somogyi units
Creatine (plasma)	0.2-0.6 mg	Lipase (serum)	0.1-1.5 units
Creatinine (plasma)	0.7-1.2 mg	Acid phosphatase (plasma)	0-1
Glucose (blood)	80-100 mg		Shinowara units
Lactate (blood)	8-12 mg	Alkaline phosphatase (plasma)	1.5-4
Pyruvate (blood)	0.4-0.8 mg		Bodansky units
Total lipids (plasma)	300-800 mg	Glutamate-oxaloacetate	
Triglycerides (plasma)	100-240 mg	transaminase (serum)	0.6-2.5 IU
Fatty acids (plasma)	5-15 mg	Glutamate-pyruvate	
Phospholipids (plasma)	140-260 mg	transaminase (serum)	0.3-2.6 IU
Lecithins (plasma)	55-180 mg	Sodium (serum)	320-340 mg
Cephalins (plasma)	2-30 mg	Potassium (serum)	17-22 mg
Sphingomyelins (plasma)	15-30 mg	Calcium (serum)	9-11 mg
Plasmalogens (plasma)	6-10 mg	Magnesium (serum)	2-2.5 mg
Total sulfate (serum)	0.03-0.06 mEq	Iron (serum)	80-160 μ g
Neutral sulfur (blood)	2-4.4 mg	Iodine (plasma)	5-10 μ g
Ethereal sulfate sulfur		Copper (serum)	100 μ g
(blood)	0.1-1.1 mg	Chloride (serum)	330-350 mg
Glutathione (blood)	25-40 mg	Inorg. phosphate (serum)	3.1-4.4 mg
Ergothioneine (blood)	10-20 mg	Bicarbonate (serum)	2.5-2.6 mEq

Blood participates in it in many ways: (i) Blood buffers neutralize acids and bases and regulate the *acid-base balance*. (ii) The hydrostatic pressure of blood and the colloid osmotic pressure of plasma proteins maintain the normal *fluid balance* between the blood and interstitial fluids. (iii) The circulation of blood distributes heat almost equally all over the body and also participates in heat loss from body surfaces, thus regulating the

body temperature. (iv) Distribution of hormones by blood all over the body also helps in homeostasis. Functions of blood in relation to respiration and excretion also assist in homeostasis.

9.3 ERYTHROCYTES

Erythrocytes of mammals do not possess the nucleus and other membrane-bound

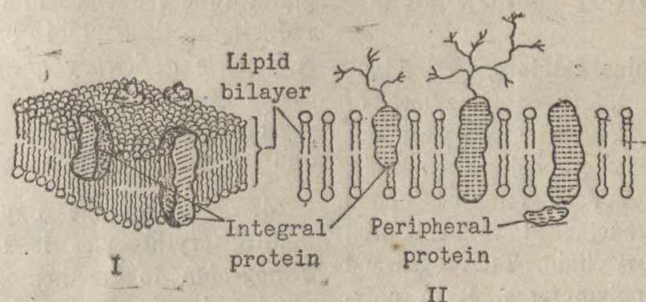


Fig. 9.1. Fluid mosaic model of erythrocyte membrane.

I: Three-dimensional view of membrane. II: Branching oligosaccharide sidechains of integral proteins project on membrane surface.

organelles. Loss of the nucleus and reduction in the cell-size as compared to lower vertebrates (diameter $6.6\text{--}9.2\text{ }\mu\text{m}$ in man and $13.7 \times 6.8\text{ }\mu\text{m}$ in chicken) increase the gas transport efficiency, particularly by increasing the surface : volume ratio. The biconcave circular disc-like shape of human erythrocyte enables it to accommodate some additional fluid by swelling without rupture and also allows the cell to be folded or partly distorted during its passage through very narrow vessels.

Composition

Erythrocytes possess a plasma membrane, about 6 nm thick and made of lipoproteins and glycoproteins. The plasma membrane consists of a fluid lipid bilayer in which proteins remain embedded in a discontinuous scattered manner. Thus a *fluid mosaic*-like structure is produced in the membrane. The lipid bilayer is made of two monolayers of lipids including phospholipids, cholesterol and its esters. Lipids constitute about 45% of the membrane. The lipids of each monolayer have their nonpolar tails directed towards the middle of the bilayer; on the contrary, their polar head groups are located at the water-adjointing surface of the respective monolayers (Fig. 9.1). Proteins form about 50% of the

membrane constituents. *Integral proteins* such as glycophorin remain deeply embedded and strongly held in the lipid bilayer; some of them extend across the entire thickness of the latter to reach both surfaces of the membrane while others penetrate into only a part of the bilayer and reach only one of its surfaces. *Peripheral proteins* such as spectrin are relatively weakly held on the inner surface of the membrane by the exposed coils of some integral proteins.

Oligosaccharide sidechains remain attached by N-glycosidic and O-glycosidic linkages to the exposed coils of some integral proteins on the outer surface of the membrane. Some other oligosaccharides are components of membrane glycosphingolipids. Oligosaccharides form about 5-7% of the membrane constituents. They are rich in N-acetylhexosamine, sialic acid, galactose, mannose and L-fucose residues. Some membrane oligosaccharides serve as *blood group antigens*.

Each human erythrocyte contains on average 280 million molecules of hemoglobin. Glucose concentration is almost similar to that of plasma. But glycogen is absent in mature erythrocytes while creatine phosphate, RNA and DNA are almost absent.

K^+ and Cl^- are the major inorganic ions; Ca^{2+} , Mg^{2+} , Na^+ , HCO_3^- and PO_4^{3-} are also present.

The cytosol contains catalase, peroxidase, superoxide dismutase, three isozymes of carbonic anhydrase, and the enzymes for glycolysis and pentose phosphate pathway. Na^+ - K^+ -dependent ATPase remains bound in the cell membrane. But mitochondrial enzymes of respiratory chain, TCA cycle and β -oxidation, DNA polymerases, RNA polymerases, and microsomal enzymes for the synthesis of proteins, fats, porphyrins, purines and cholesterol are absent due to the absence of cell organelles.

Metabolism

In absence of organelles and their enzymes, β -oxidation, TCA cycle and synthesis of proteins, nucleic acids, cholesterol and hemoglobin cannot occur in mature erythrocytes. Consequently, the metabolic need for ATP is much lower in mature RBC than in other cells. The high-energy bonds of ATP are required mainly for running the sodium pump and for maintaining ionic balance and structural integrity. ATP is generated by glycolysis in erythrocytes.

1. *Entry of glucose into human erythrocytes depends on a carrier-mediated passive transport.* D-Glucose is transported by a carrier protein across the plasma membrane and down the inward concentration gradient of the sugar, but never against the concentration gradient. The transport requires no metabolic energy.

2. *Glycolysis is the main energy-producing pathway in mature erythrocytes.* These cells do not contain glycogen. During glycolysis, cytosol enzymes catabolize glucose itself to lactate. Lactate is mainly released in the blood, because it cannot be oxidized in these cells through the TCA cycle.

3. Some glucose is also metabolized through the *pentose phosphate pathway*.

NADPH formed in this pathway is utilized by glutathione reductase and also by an enzyme reducing methemoglobin back to hemoglobin. A genetic deficiency of *glucose-6-phosphate dehydrogenase* of this pathway increases erythrocyte fragility to cause hemolytic anemia and jaundice in the newborn.

4. Most of the oxygen consumed in mature erythrocytes is spent in *oxidizing hemoglobin* to methemoglobin; molecular oxygen is simultaneously changed to the *superoxide radical* O_2^- by univalent reduction. A cytoplasmic copper-zinc enzyme, *superoxide dismutase*, immediately transfers electrons from the superoxide radical to H^+ to produce O_2 and H_2O_2 : $2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$. H_2O_2 is subsequently reduced to H_2O by glutathione (GSH) and *glutathione peroxidase*, a selenium-containing enzyme. The latter thereby protects hemoglobin against oxidation, by destroying peroxides: $H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$. Oxidized glutathione (GSSG) is immediately reconverted to glutathione by *glutathione reductase*, utilizing NADPH. $GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$.

5. Any methemoglobin produced is immediately reduced to hemoglobin by either an NADH-dependent heme enzyme called *methemoglobin reductase* or an NADPH-dependent reducing enzyme. A congenital deficiency of methemoglobin reductase prevents the reduction of methemoglobin to hemoglobin; the consequent rise in the blood methemoglobin level (*familial methemoglobinemia*) produces a bluish hue of skin and mucosa (*cyanosis*).

6. A metabolic bypass, called the *Rapoport-Luebering cycle* (RL cycle), occurs in erythrocytes as a supplement to glycolysis. It reduces the genesis of high-energy bonds. During glycolysis, glucose is converted to 1,3-diphosphoglyceric acid; the latter is next changed by phosphoglycerate kinase to 3-phos-

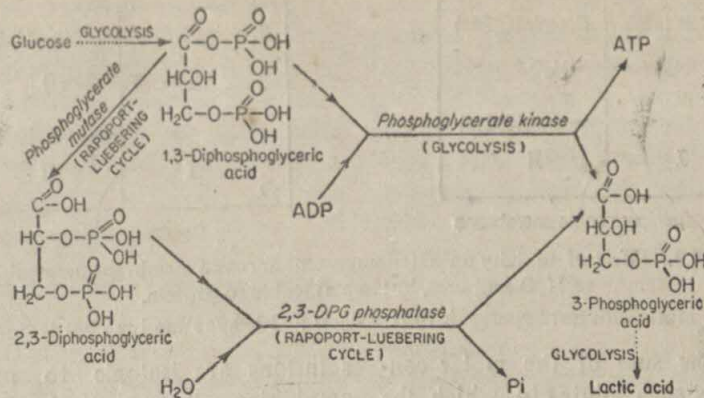


Fig. 9.2. Rapoport-Luebering cycle.

phosphoglyceric acid with the production of a high-energy phosphate bond. But in the RL cycle (Fig. 9.2), 1,3-diphosphoglyceric acid is changed by phosphoglycerate mutase to 2,3-diphosphoglyceric acid (2,3-DPG); the latter is hydrolyzed by 2,3-DPG phosphatase to yield 3-phosphoglyceric acid and Pi without the formation of any high-energy bond.

2,3-DPG gets bound to the β chains of hemoglobin to reduce its O_2 affinity—acclimatization at high altitudes increases 2,3-DPG significantly in erythrocytes and helps to release more O_2 from oxyhemoglobin. Increase in 2,3-DPG is observed in a *hereditary deficiency of pyruvate kinase*, a glycolytic enzyme of red cells; the defect is related to an autosomal recessive gene. A *genetic deficiency of the red cell hexokinase*, another glycolytic enzyme, lowers the 2,3-DPG content of RBC and interferes with the delivery of O_2 from oxyhemoglobin.

9.4 CRENATION, HEMOLYSIS AND ISOTONIC SALINE

Plasma membrane is *semipermeable*—it permits water and a few solutes to pass freely through it, but many other solutes including proteins and some electrolytes either cannot cross it or do so with extreme difficulty.

Osmosis takes place across the plasma membrane whenever the intracellular and extracellular fluids differ in concentration of the nondiffusible or less diffusible solutes.

When a solution is separated from the pure solvent by a perfectly semipermeable membrane which allows the solvent particles but none of the solute particles to pass through, the osmotic pressure of the solution is proportional to the sum of the molar concentrations of all its solutes. Moreover, the osmotic pressure of this solution is identical with that of any other solution, having the same total molar concentration of solutes and remaining separated from the solvent by a perfectly semipermeable membrane. Solutions with such an identical osmotic pressure are called *isosmotic solutions*. A solution is called *hyperosmotic* or *hyposmotic* in comparison to another when the former has respectively a higher or lower osmotic pressure than the latter. But the plasma membrane is not perfectly semipermeable—it allows the diffusion of water and some solutes while preventing the diffusion of some other solutes. Thus, an extracellular fluid, separated from the cytosol by the plasma membrane, exhibits only a fraction of its total osmotic pressure—this fraction of the total osmotic pressure is called the *tonicity* of the solution and is

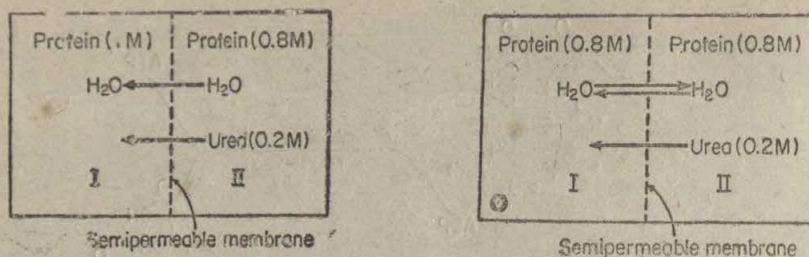


Fig. 9.3. Effect of tonicity on water movement across a membrane permeable to H_2O and urea, but impermeable to protein.

Left: I is iso-motic with but hypertonic to II. Right: I is hypotonic to but isotonic with II.

proportional to the sum of the molar concentrations of only such solutes to which the membrane is impermeable. Two solutions of identical tonicity are called *isotonic solutions* though they are not necessarily isosmotic. For example, if the plasma membrane, permeable to water and urea but not to proteins, separates an 1 M protein solution from another solution of 0.8 M protein and 0.2 M urea, the two solutions are isosmotic but not isotonic—the tonicity is higher in the first solution than in the second, because it depends on the protein concentration alone. On the other hand, the first solution would have a lower osmotic pressure but an identical tonicity with the second if the protein concentration is lowered to 0.8 M in the former (Fig. 9.3). The osmotic flow of water depends on the difference in tonicity across the membrane. Animal cells remain unaltered in size, shape and fluid content, when in an isotonic solution, because of no net movement of water across the plasma membrane. In any species, extracellular fluids are normally almost isotonic to the cell—0.9% and 0.65% NaCl

solutions are isotonic to mammalian and amphibian cells respectively (Table 9.2).

Hypertonic and hypotonic solutions have respectively higher and lower tonicities than the solution under reference. If erythrocytes are placed in a hypotonic solution (say, 0.3% NaCl), water passes into the cells from the solution by osmosis (endosmosis), making the cells swell. This osmotic inflow of water may ultimately distend the cell to such an extent that its plasma membrane gets leaky and becomes permeable even to macromolecules. Consequently, hemoglobin dissolved in the intracellular fluid passes out through the membrane to the surrounding medium (*hemolysis*). The red cell, now devoid of hemoglobin but still possessing a seemingly undamaged plasma membrane is called an *erythrocyte ghost*. It is very suitable for the study of membrane composition and properties. If the ghost cell is placed in an isotonic solution again, its plasma membrane regains its semipermeability.

If erythrocytes are placed in a hypertonic solution (say, 1.2% NaCl), water is osmotically

TABLE 9.2. *Compositions of isotonic salines (g dl⁻¹).*

	NaCl	KCl	CaCl ₂	MgCl ₂	NaHCO ₃	NaH ₂ PO ₄	Glucose
Mammalian Ringer	0.86	0.03	0.033	—	—	—	—
Tyrode	0.80	0.02	0.02	0.005	0.1	0.004	0.1
Locke	0.90	0.042	0.024	—	0.02	—	0.1
Dale	0.90	0.042	0.024	0.002	—	—	—
Amphibian Ringer	0.65	0.014	0.012	—	0.02	0.005	—
Earthworm Ringer	0.60	0.012	0.02	—	0.01	0.001	—

drawn out from them (exosmosis), making the cells shrink (*crenation*). Normally, erythrocytes have a slightly higher tonicity than the plasma due to a slightly higher intracellular concentration of electrolyte ions; so these cells normally remain well-distended with fluid in blood.

Erythrocyte fragility test

Damages or defects of erythrocyte membrane, due to genetic disorders or deficiencies of Se or vitamin E, may increase the fragility of those cells, leading to hemolysis even at the normal osmotic distension. To estimate the osmotic fragility of erythrocytes, the patient's blood is added to different saline concentrations ranging from 0.1 to 0.9% and the concentrations, at which hemolysis is respectively started and completed, are noted. Normally, these are 0.48% and 0.33% NaCl respectively.

9.5 LEUCOCYTES

Composition: K^+ is the major cation, with lesser amounts of Zn^{2+} , Ca^{2+} , Mg^{2+} , etc. Inorganic anions include Cl^- , HCO_3^- and phosphates. Zn^{2+} concentration averages 32 fg per million WBC and far exceeds that of RBC; but the Zn^{2+} -enzyme carbonic anhydrase is absent in leucocytes. Zn^{2+} occurs mainly in the cytoplasmic superoxide dismutase along with Cu^{2+} .

Neutrophils possess three types of cytoplasmic granules. Primary or azurophil granules contain the enzymes myeloperoxidase and lysozyme as well as cationic proteins. Secondary or specific granules contain lysozyme, an iron-binding substance called lactoferrin, and a B_{12} -binding protein. Tertiary granules are lysosomes storing acid hydrolases in their lumen. Eosinophils and basophils also carry azurophil granules and specific granules. Basophils have metachromatic granules storing heparin, histamine, serotonin, platelet activating factor and neutrophil and

eosinophil chemotactic factors. Secondary granules of eosinophils store cationic proteins, arylsulfatase B, phospholipase D and histaminase. Monocytes do not possess secondary granules, but have lysosomal granules. Granulocytes contain glycogen in abundance for glycolysis during phagocytosis. DNA and RNA polymerases, enzymes for glycolysis, pentose phosphate pathway and protein synthesis occur in leucocytes. Lymphocytes contain immunoglobulins. Some antigens called *human leucocyte antigens (HLA)* occur on leucocyte membranes.

Biochemical changes in phagocytosis

1. Phagocytosis results from contractions of cytoplasmic microfilaments, made of contractile actin-like proteins.

2. After the phagocytic vesicle or phagosome has been formed, granules of the phagocyte fuse with the phagosome and drain their contents into it. The contents of granules then act on the phagocytosed material.

3. Both glycolysis and the reactions of pentose phosphate pathway increase manifold during phagocytosis, the former providing the additional energy and the latter generating NADPH for superoxide formation.

4. Phagocytosis is accompanied by a steep and manifold increase in the oxygen consumption (*respiratory burst*) by the phagocyte. This oxygen is utilized in forming superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide and free hydroxyl radical ($\cdot OH$); all these are very effective in killing the phagocytosed microbe.

(a) Superoxide anion (O_2^-) is produced by the reduction of molecular O_2 with the electron donated by NADPH and with the help of a membrane flavoprotein called *NADPH oxidase*. The latter acts with cytochrome b_{558} of the membrane. A tissue substrate (S) is reduced simultaneously with O_2 : $NADPH + S + O_2 \rightarrow NADP^+ + SH + O_2^-$.

(b) The superoxide is spontaneously changed to singlet oxygen ($^1\text{O}_2$) and H_2O_2 in the phagosome: $2\text{O}_2^- + 2\text{H}^+ \rightarrow ^1\text{O}_2 + \text{H}_2\text{O}_2$.

(c) The superoxide also reacts with H_2O_2 in presence of *lactoferrin* and iron to form singlet oxygen, hydroxyl ion and free hydroxyl radical ($\cdot\text{OH}$): $\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow ^1\text{O}_2 + \cdot\text{OH} + \text{OH}^-$. This reaction is called the *Haber-Weiss reaction*.

(d) *Myeloperoxidase*, a heme enzyme of azurophil granules, catalyzes the oxidation of halide ions to active halides like hypochlorite ions (ClO^-). The latter may destroy the phagocytosed microbe by halogenating its cell wall or by decarboxylating amino acids to toxic aldehydes: $\text{H}_2\text{O}_2 + \text{Cl}^- \rightarrow \text{H}_2\text{O} + \text{ClO}^-$.

(e) Hypohalites may react with H_2O_2 to form singlet oxygen and halide ions: $\text{ClO}^- + \text{H}_2\text{O}_2 \rightarrow \text{Cl}^- + ^1\text{O}_2 + \text{H}_2\text{O}$.

(f) Any superoxide, leaking into the cytoplasm from the phagosome, is reduced by a Cu^{2+} - Zn^{2+} enzyme called *superoxide dismutase* of the cytoplasm: $2\text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$. Any H_2O_2 , leaking into the cytoplasm is destroyed by catalase in the cytoplasm: $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$. Some H_2O_2 is also reduced in the cytoplasm with the help of glutathione (GSH) and *glutathione peroxidase*: $\text{H}_2\text{O}_2 + 2\text{GSH} \rightarrow 2\text{H}_2\text{O} + \text{GSSG}$; the oxidized glutathione (GSSG) from this reaction is reduced back to GSH by NADPH and *glutathione reductase*: $\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADP}^+$. As the phagosome membrane is impermeable to superoxide dismutase, catalase and glutathione peroxidase, they do not interfere with the actions of O_2^- and H_2O_2 in the phagosome.

5. Some oxygen-independent processes also take place in the phagosome due to chemical substances drained into the latter from the cytoplasmic granules.

(a) *Lysozyme* hydrolyzes β -1,4-glycosidic bonds between N-acetylhexosamine and

N-acetylmuramic acid residues in the cell wall peptidoglycans of Gram-positive bacteria, causing their lysis.

(b) *Lactoferrin* binds iron to make it unavailable to proliferating bacteria, stopping their multiplication.

(c) *Acid hydrolases* from lysosomes hydrolyze bacterial nucleic acids, proteins, lipids and oligosaccharides at a low pH inside the phagosome.

(d) *Cationic proteins* of azurophil granules damage bacterial membranes by binding electrostatically with anionic membrane groups and also by exerting proteolytic actions.

9.6 PLASMA PROTEINS

Plasma proteins are variously classified according to the method of separation. Each of the three major classes, viz., serum albumin, serum globulin and fibrinogen, is a mixture of many types of proteins of various molecular sizes.

Separation and fractionation

1. **Salting out**: Particularly near their isoelectric pH, proteins are effectively precipitated by relatively high concentrations of electrolyte salts. Electrolyte ions (i) dehydrate the shell of solvent (solvation layer) around the protein particles by attracting the polar water molecules, and (ii) alter or discharge the double-layer of electric charges around those particles—both these effects bring protein particles together into aggregates, heavy enough to precipitate. Salting out by electrolytes produces five main fractions of plasma proteins with an ascending order of solubility in water, viz., fibrinogen, euglobulin, pseudoglobulins I and II and albumin.

TABLE 9.3. *Salting out of plasma proteins.*

<i>Electrolyte used</i>	<i>Precipitating concentration</i>	<i>Fraction precipitated</i>
(NH ₄) ₂ SO ₄	0.25 saturation	fibrinogen
	0.33 saturation	euglobulins
	0.40 saturation	pseudoglobulin I
	0.46 saturation	pseudoglobulin II
	full saturation	albumin
Na ₂ SO ₄	13.5%	euglobulins
(Howe's method)	17.4%	pseudoglobulin I
	21.5%	pseudoglobulin II

2. **Precipitation by organic solvents:** At different ionic strengths, temperatures and pHs, variations in solvent concentrations may differentially precipitate the plasma proteins. *Dielectric constant* of a substance is a measure of its capacity to oppose the electrostatic attraction between antagonistic charges separated by it—the high dielectric constant of water helps in keeping protein ions in solution by dispersing them in plasma. On addition of liquids with low dielectric constants (e.g., ethanol or acetone) to plasma, the overall dielectric constant is depressed, consequently increasing the interionic attraction between the protein ions and leading to their aggregation and precipitation. Such alterations of dielectric constants of the solution form one basis of *Cohn's method* for separating plasma proteins—different ethanol concentrations (8-40%), pHs (4.5-7.2), temperatures (-3 to -5°C) and ionic strengths (0.01-0.14) are used to separate plasma proteins into 6 fractions. Fraction I is obtained by treating plasma with 8-10% ethanol at pH 7.2 and -3°C. It is rich in fibrinogen. Progressive reduction in pH and enhancement of ethanol concentration precipitate other fractions. Fraction II is rich in γ -globulins, III in α - and β -globulins, isoagglutinins and prothrombin, IV in α - and β -globulins again, and V in albumin.

3. **Free-boundary electrophoresis:** This method has been described on pages 69-70.

Tiselius first used this method for separating human plasma proteins into 5 fractions with progressively decreasing order of electrophoretic mobility (Fig. 3.34). Each protein fraction produced a peak in the plasma electrophoretic pattern. Albumin and fibrinogen were each shown to consist of proteins having similar MW and ionic charges as they produced single sharp peaks; globulin produced several broad peaks which indicated the presence of several ionic species such as α , β and γ globulins.

4. **Zone electrophoresis:** The method of *paper electrophoresis* (see page 70) has been used for fractionation and precise estimation of small amounts of plasma proteins for diagnostic purposes. *Gel electrophoresis* (see pages 70-71) has also been used for the same purpose. About 20 fractions of plasma proteins have been separated by gel electrophoresis using starch gel block.

5. **Immunoelectrophoresis:** Plasma proteins have been separated by this method (see pages 71-72), depending on their electrophoretic migration and antigenicity. The electrophoretic separation is done by gel electrophoresis on an agar gel block while the separated protein fractions are precipitated on the block by using an immune antiserum containing antibodies against those proteins (Fig. 3.37).

6. **Ultracentrifugation:** This method, described on pages 66-67, has also been used sometimes for the fractionation of plasma proteins and determination of their sedimentation coefficients and molecular weights.

7. **Chromatography:** Chromatographic methods such as *ion exchange chromatography* have been used for fractionation of plasma proteins. These methods have been described on pages 67-69.

Classification

1. **Prealbumin:** It is an oligomeric protein, formed of four identical peptide

monomers, and occupies a zone ahead of albumin in the electrophoretic pattern (vide Table 9.4).

2. **Albumin** : It is the major plasma protein in mammals, birds and adult amphibians, and occupies a zone between prealbumin and globulins in the electrophoretic pattern. It is also present in Cohn's fraction V. It forms about 54-60% of the total plasma proteins. Its ellipsoid molecule (15 nm $\times$ 3.8 nm) is a single, folded, low α -helical polypeptide of about 580 amino acid residues. Several intrachain S-S bonds fold the peptide chain into three domains, each with several sub-domains. Due to its considerably low isoelectric pH, albumin bears 18 negative charges at pH 7.4 ; this property as well as its relatively low MW explains its rapid electrophoretic migration. It is quite soluble in plasma at pH 7.4, but contributes relatively less viscosity to plasma than globulins. Its relatively small molecular size, high concentration, and collection of water molecules around its anionic hydrophilic groups give a considerable osmotic pressure to plasma.

Elasmobranchs, cyclostomes, primitive teleosts and young amphibians like tadpoles have little or no albumin in the blood.

3. **Globulins** : They have larger and more asymmetric molecules than albumin and constitute about 44-45% of total plasma proteins. Three globulin fractions may be separated by salting out, viz., *euglobulin* which is insoluble in pure water but soluble in dilute neutral salt solutions, and *pseudoglobulins* I and II which are soluble in pure water itself ; all three fractions are mixtures of many proteins. Electrophoresis yields many fractions like α_1 , α_2 , β_1 , β_2 and γ globulins, each being a mixture of many proteins (vide Table 9.4) ; α_1 , α_2 and β globulins constitute respectively 5.3%, 8.7% and 13.4% of total plasma proteins. Many of the α and β globulins are glycoproteins or mucoproteins with oligosaccharide prosthetic groups

composed of hexosamines, hexoses and sialic acids. Some of the globulins also contain metal ions in their prosthetic groups. The α and β globulins include *lipoproteins* which migrate with them and have lipid components composed of triglycerides, phospholipids, free fatty acids, and free and esterified cholesterol. The lipid component remains bound by hydrophobic bonds with proteins, called *apolipoproteins*, rich in nonpolar amino acid residues. Several types of apolipoproteins like apo-AI, apo-AII, apo-B, apo-CI, apo-CII and apo-CIII have been identified. Lipoproteins have densities ranging from <0.95 to >1.21 ; the density varies inversely with the diameter and the lipid content of the lipoprotein. Very high density lipoproteins (VHDL) and high density lipoproteins (HDL) migrate with α globulins, carry apo-AI and apo-AII apolipoproteins and constitute about 3% of total plasma proteins ; low density lipoproteins (LDL) and very low density lipoproteins (VLDL) migrate with β globulins, carry apo-B, apo-CI, apo-CII and apo CIII apolipoproteins and represent 5% of total plasma proteins. VHDL, HDL, LDL and VLDL contain about 45%, 66%, 77% and 92% lipids, respectively.

γ -Globulins consist mainly of *immunoglobulins* which act as antibodies. They will be discussed later in this chapter.

4. **Fibrinogen** : This glycoprotein constitutes about 4% of total plasma proteins and has large ellipsoid molecules (46 nm $\times$ 9 nm) exerting considerable viscosity in solution (Table 9.4). The molecule is formed of three interconnected globular units, the side-globular units, the side-globular units, the side-globules being larger than the central one. The protein part of the molecule is made of 6 peptide chains, viz., pairs of A α , B β and γ peptides joined by S-S linkages. The β -amide-N in the sidechain of an asparagine residue in each B β or γ chain is bound by an N-glycosidic linkage to a heteroglycan of D-mannose, D-galactose, sialic acids and N-acetylhexosamines. The A and

TABLE 9.4. *Some major human plasma proteins.* (1 kdal = 10^3 daltons.)

Protein	Concentration (mg dl ⁻¹)	MW (kdal)	Sedimentation coefficient (S)	Isoelectric pH	Carbohydrate %
Prealbumin	20-30	55	3.9	4.7	0
Albumin	2800-4500	69	4.6	4.9	0
Globulins	1500-2500				
α_1 Globulins	200-500				
Retinol-binding	3-5	21	2.3		0
Orosomucoid	50-150	40	3.5	2.7	40
Transcortin	3-4	52	3.6		25
Antitrypsin	100-300	54	3.4	4.8	11
Thyroxine-binding	1-2	58	3.9		12
α_2 Globulins	300-1000				
Ceruloplasmin	20-50	150	7.1	4.4	6
Haptoglobulins	200-800	100-400	4.5-7.5	4.1	20
Macroglobulin	150-350	730	19.5	5.4	10
β Globulins	500-1100				
Hemopexin	40-80	57	4.8		20
Transferrin	40-60	85	5.4	5.5	5
γ Globulins	500-1500	150	7.0	6.2-7.5	2-5
Fibrinogen	200-500	340		5.8	5
α_1 Lipoproteins	300-450	200-400		(lipid content = 40-70%)	
β_1 Lipoproteins	300-450	2000-20000		(lipid content = 70-90%)	

α portions of each $A\alpha$ chain as well as the B and β portions of each $B\beta$ chain are joined by Arg-Gly peptide bonds (Fig. 9.6) which are specifically hydrolyzed by thrombin to cleave fibrinogen to fibrin during blood coagulation. In spite of its large fibrous structure, its molecules are prevented from aggregation and kept in solution in plasma by intermolecular electrostatic repulsions due to strong negative charges at their terminals. These negative charges result from the anionic sidechains of many glutamate and aspartate residues of the A portion of $A\alpha$ and the B portion of $B\beta$ chains as also anionic tyrosine-O-sulfate residues of the B portion of $B\beta$ chains.

Origin

(a) *Serum albumin* is formed in the mammalian liver from amino acids of dietary

proteins, particularly those of high biological values like milk proteins, liver, flesh proteins and egg-white. Low-protein diets, deficiency of essential amino acids, hepatectomy, hepatitis or hepatic cirrhosis may considerably lower the serum albumin level as well as the total plasma protein (*hypoproteinemia*). An inborn defect in the ribosomal synthesis of serum albumin may produce very low serum albumin levels (*hereditary analbuminemia*). In experimental animals, Whipple's *plasmapheresis* has been used to reduce the serum albumin level—the blood is taken out in portions and the washed corpuscles, suspended in Ringer's solution, are returned into the circulation; the serum albumin level is rapidly restored provided the animal has a healthy liver and takes a high-protein diet.

(b) *Fibrinogen* is also synthesized in the liver. Injury, failure or removal of liver

lowers the plasma fibrinogen. *Afibrinogenemia* is an acute inborn deficiency of fibrinogen due to a recessive genetic defect in its synthesis. The three genes transcribing the RNAs for $\text{A}\alpha$, $\text{B}\beta$ and γ chains of fibrinogen in the hepatocyte nucleus are genetically linked. They are subject to coordinate induction and repression.

(c) α and β globulins are synthesized in the liver. The hepatic synthesis of several pro-coagulation globulins, including prothrombin and Stuart factor, involves the incorporation of an additional carboxyl group at the γ -C of specific glutamate residues of those proteins with the help of vitamin K. So, in vitamin K deficiency, these coagulation factors are deficient in the plasma and blood coagulation is delayed.

(d) *Immunoglobulins* are synthesized after birth by B lymphocytes and plasma cells in the lymphoid tissues and plasma, but are released in the plasma by the plasma cells alone. The importance of lymphoid tissues in immunoglobulin synthesis is indicated by abnormally high serum immunoglobulin in *Lymphogranuloma venereum* with increased lymphoid activity, as also by the poor capacity of immunoglobulin synthesis in invertebrates and cyclostomes lacking in lymphoid tissues.

(e) *Lipoproteins* are largely synthesized in the liver. The apolipoproteins, synthesized by hepatic ribosomes, are combined with lipids (mainly triglycerides) to form very low density lipoproteins (VLDL) which are released in the plasma. On the other hand, the high density lipoproteins (HDL) of plasma are synthesized by the hepatic and intestinal cells by combining specific apolipoproteins with lipids, rich in cholesterol esters and phospholipids. Extrahepatic tissues like adipose tissue remove much of the triglycerides from the circulating VLDL by lipolysis to change the latter to the low density lipoproteins (LDL), richer in cholesterol esters than in triglycerides.

Nutritional factors in plasma protein synthesis: Amino acids, obtained from dietary proteins, form the chief source for plasma protein synthesis. So, the latter is directly influenced by the nutritional quality and quantity of dietary proteins. This is substantiated by: (i) high incidence of *hypoproteinemia* (low plasma protein) in case of a deficiency of any essential amino acid, (ii) higher efficacy of dietary proteins of high biological value in restoring the plasma protein level in dogs after plasmapheresis and (iii) failure of the rise in antibody titre in response to antigens in rats kept on diets poor in tryptophan and phenylalanine.

Functions

1. Transport :

Albumin and α and β globulins combine with substances of low solubilities (e.g., cholesterol, triglycerides, fat-soluble vitamins and bilirubin) to form more soluble complexes easily transportable in the plasma. Metal ions and small molecules such as heme, hemoglobin and thyroxine are also transported in the plasma in combination with large plasma proteins to prevent their urinary loss by filtration. Some examples of substances transported by different plasma proteins are cited in Table 9.5.

2. Colloid osmotic pressure :

Although only 0.5% of the total osmotic pressure of plasma is exerted by the colloidal plasma proteins, this is of great importance in retaining fluid in blood. In human plasma, albumin and globulins are responsible for osmotic pressures of 20-28 and 3-4 mm Hg respectively. The far greater osmotic effect of albumin is due to its higher concentration, lower MW and the attraction exerted by its negatively charged hydrophilic groups on the neighbouring water molecules. The osmotic pressure of plasma proteins exceeds that of the tissue fluid proteins by 15-20 mm Hg; this is called the *effective colloid osmotic*

TABLE 9.5. *Plasma proteins in transport.*

<i>Plasma proteins</i>	<i>Substances transported</i>
Prealbumin	Thyroxine, retinol
Albumin	Aldosterone, bilirubin, free fatty acids, Ca^{2+} , naphthoquinones, penicillin, salicylates, sulfonamides, Zn^{2+}
α_1 Globulins	
Retinol-binding globulin	Retinol
Thyroxine-binding globulin	Thyroxine
Transcortin	Cortisol, corticosterone
α_2 Globulins	
Ceruloplasmin	Cu^{2+} , Cu^+
Haptoglobulins	Hemoglobin
β Globulins	
Hemopexin	Heme
Transcortin	Cobalamins
Transferrin	Fe^{3+} , (Cu^{2+} , Zn^{2+})
Transmanganin	Mn^{2+}
Lipoproteins	Triglycerides, phospholipids, cholesterol, cholesterol esters, free fatty acids

pressure of plasma and tends to draw water from tissue fluids to plasma.

According to *Starling's hypothesis*, the effective hydrostatic pressure of blood exceeds the effective osmotic pressure of plasma by 7-12 mm Hg at the arterial end of capillaries and filters out some fluid from blood to tissue spaces. At the venous end of capillaries, the effective hydrostatic pressure is lower than the effective osmotic pressure of plasma by 7-10 mm Hg so that some fluid is drawn back from tissue fluids to blood. Thus, the colloidal osmotic pressure of plasma proteins maintains the circulating fluid volume and the fluid balance between plasma and tissue fluids.

Commensurate with its importance in the colloid osmotic pressure of plasma, albumin concentration has increased considerably in mammals and higher vertebrates, adapted to dehydrating terrestrial habitats; elasmobranchs, cyclostomes and many teleosts have little or no serum albumin.

3. Blood coagulation :

Blood is coagulated by the well-coordinated actions of fibrinogen and several globulins like prothrombin, antihemophilic factor, proconvertin, proaccelerin, Christmas and Stuart factors and plasma thromboplastin antecedent. Intravascular clotting is prevented by another globulin, antithrombin III.

4. Body defence and immunity :

When an antigen enters the body, a specific type of immunoglobulin (antibody) is secreted by plasma cells and may react with the corresponding antigen to form large aggregates of antigen-antibody complexes. These are precipitated and subsequently removed by phagocytosis or enzymatic dissolution. Immunoglobulins, bound to the membrane antigens of a foreign cell, help to bind some other globulins, called *complements*, with the same membrane and initiate their activation into proteases; the latter cause lysis of the antigenic cell, attract leucocytes to that cell,

promote its being phagocytosed and also produce inflammations and allergies.

5. Blood buffers :

Plasma proteins constitute the protein buffers of plasma, averaging 13 mEq litre⁻¹ in concentration.

6. Viscosity of plasma :

Plasma viscosity normally ranges from 15 to 20 millipoises at 20°C and is mainly due to large elongated plasma protein molecules. Albumin with smaller and more globular molecules contributes less viscosity than globulins and fibrinogen which have larger and more ellipsoid molecules. Plasma viscosity exerted by plasma proteins helps in hemodynamics by offering a resistance against blood flow and by reducing the tendency of turbulence inside vessels.

7. Enzyme activities :

Ceruloplasmin, a blue α_2 -globulin containing copper, serves as *serum ferroxidase* to catalyze the oxidation of Fe^{2+} to Fe^{3+} in plasma. C1r, C1s and C3 are globulin proenzymes and form *serine proteases of the complement system* on activation. Globulins like prothrombin, Christmas factor, Stuart factor and plasma fibrin stabilizing factor are proenzymes which, on activation, form *proteases for blood coagulation*. The globulin plasminogen acts, on activation, as a *protease for fibrinolysis*. But most plasma enzymes like acid and alkaline phosphatases, serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) come from other tissues and may not possess normal roles in plasma, although their plasma titres are helpful in clinical diagnosis (see page 112).

Some serum globulins like antithrombin III, α_2 -macroglobulin, α_1 -antitrypsin and inter- α -trypsin inhibitor *inhibit serine proteases*, including coagulating and fibrinolytic enzymes and proteases from extravascular sources.

8. Source of tissue proteins :

Tissue cells pinocytize both albumin and globulins and utilize their amino acids for building specific tissue proteins.

Genetic deficiencies

1. *Analbuminemia* : This is an inherited disorder characterized by very low serum albumin levels due to an inborn defect in albumin synthesis. Plasma levels of lipids and lipoproteins are also raised indicating a secondary defect in lipid transport.

2. *Afibrinogenemia* : Vide 9.7.

3. *Hypoproteinaemia* : Vide 9.7.

4. *Bruton's agammaglobulinemia* : This inherited disease is characterized by the absence or very low levels of γ -globulins, absence of circulating plasma cells and lack of humoral immunity against bacterial infections. The defect is inherited as an X-linked recessive trait and involves a failure of differentiation of B lymphocytes into plasma cells.

5. *Abetalipoproteinemia and hypobetalipoproteinemia* : These are caused by an inborn failure of B apolipoprotein synthesis leading to the absence or very low levels of VLDL, LDL and chylomicrons in the blood. This leads to reduced absorption and mobilization of fat, fatty liver and myelin degeneration, because VLDL and chylomicrons are involved in fat mobilization.

6. *Tangier disease* : This is caused by a genetic failure of apo-HDL (e.g., CII apolipoprotein) synthesis and is characterized by poor plasma levels of HDL. As HDL transports mainly phospholipids, cholesterol and cholesterol esters and only small amounts of triglycerides, the disease lowers the plasma levels of cholesterol and phospholipids, but not of triglycerides.

7. *Hemophilias, parahemophilia, Hageman trait* : Vide 9.7.

Clinical changes in plasma proteins

Serum albumin level may fall (*hypoalbuminemia*) in several pathological conditions: (a) due to urinary loss of plasma proteins in nephrosis and glomerulonephritis, (b) due to protein malnutrition in kwashiorkor and marasmus, (c) due to a failure of hepatic synthesis in liver cirrhosis, (d) due to loss of blood proteins in case of trauma and burns, and (e) due to excessive loss of proteins into the alimentary canal—the last factor causes a simultaneous lowering of all the plasma proteins and is known as *ideopathic hypoproteinemia*. The generalized edema, characteristic of kwashiorkor, is largely due to hypoalbuminemia. Edema is absent in marasmus because hypoalbuminemia is much less acute in this protein deficiency disease. On the other hand, dehydration may cause *hyperalbuminemia* in consequence of hemoconcentration.

Inflammations and tissue destruction, associated with trauma, infections, advanced tuberculosis, later stages of malignancies, rheumatoid arthritis, etc., may raise serum α -globulins, particularly orosomucoid, α_1 -antitrypsin and a β -globulin called *C-reactive protein* (MW 118 kdal). β -Globulins as well as lipoproteins are elevated in hyperlipidemias characterized by accumulation of lipids in the plasma.

Elevation of the γ -globulin level is called *hypergammaglobulinemia*. It is observed in multiple myeloma with malignant proliferation of plasma cells. Abnormal globulins with low MW, called *Bence-Jones proteins*, are also found in the serum of multiple myeloma patients.

Monoclonal myopathies are characterized by the elevation of a single specific species of immunoglobulins of homologous composition.

9.7 COAGULATION OF BLOOD

Hemostasis is an arrest of bleeding. It may be carried out in three stages in mammals:

- (a) *local vasoconstriction* of the injured vessel;
- (b) formation of a *white thrombus* by the trapping and aggregation of platelets by fibrin threads at the injured spot;
- (c) formation of a *red thrombus* by the trapping of blood cells in a network of fibrin threads, and effective sealing of the injured vessel.

Role of platelets

With the help of a glycoprotein called von Willebrand factor from endothelial cells, platelets bind with exposed collagen fibres at the rough electronegative surface of the injured tissue. Such adherence of platelets to collagen fibres leads to the hydrolysis of their membrane phospholipids into diacylglycerol. The latter triggers changes in the shape of platelets, stimulates formation of pseudopodia and causes release of the following substances from their granules into the plasma. (i) *ADP*, released from platelet granules, enhances Ca^{2+} influx into the platelet, leading to an increased aggregation of platelets and a release of their granular contents. (ii) *von Willebrand factor* (MW > 200 kdal) released from platelets, promotes the adherence of platelets to the injured tissue and the action of factor VIII in the intrinsic pathway of coagulation (see below). (iii) *Thromboxane A_2* from the platelet promotes local vasoconstriction, platelet aggregation, Ca^{2+} influx into platelets, hydrolysis of membrane phospholipids into diacylglycerol, and a consequent release of materials from the platelet granules. (iv) *Serotonin* from platelet granules causes local vasoconstriction. (v) *Platelet accelerator factor II* may catalyze the conversion of fibrinogen to fibrin. (vi) *Fibrin stabilizing factor* catalyzes the formation of the final fibrin I polymer. (vii) *Thrombosthenin* is a contractile protein which helps in clot retraction. Besides, *anionic phospholipids* on the exposed inner surface of disrupted platelet membranes get bridged by Ca^{2+} ions to the anionic Glu (γ -carboxyglutamate) residues of several coagulation factors

TABLE 9.6. *Blood coagulation factors.*

Factor	Synonyms	Oligosaccharide part	MW (kdal)	Activated product	Inborn error
I	Fibrinogen	5%	*340	Fibrin	Afibrinogenemia
II	Prothrombin	4.3%	*72	Thrombin	Hypoprothrombinemia
III	Tissue thromboplastin	—	—	—	—
IV	Ca ²⁺	—	—	—	—
V	Proaccelerin, Ac-globulin	—	†350	Va	Parahemophilia
VII	Proconvertin	—	†45.5	VIIa	Factor VII deficiency
VIII	Antihemophilic factor, antihemophilic globulin	5%	*1100	VIIIa	Hemophilia A
IX	Christmas factor, plasma thromboplastin component	25%	†55.4	IXa	Hemophilia B
X	Stuart factor, Stuart-Power factor	10%	†55	Xa	Stuart factor deficiency
XI	Plasma thromboplastin antecedent	12%	*160	XIa	Hemophilia C
XII	Hageman factor	15%	†74	XIIa	Hageman trait
XIII	Fibrin-stabilizing or Laki-Lorand factor	2.5%	†150, *320	XIIIa	Fibrin-stabilizing factor deficiency

* in human plasma

‡ in human platelets

† in bovine plasma

such as prothrombin and factors VII and X, thus concentrating them on the membrane and enhancing their activation and action in blood coagulation. Such anionic phospholipids of the platelet membrane have often been called *platelet thromboplastic factors*.

Mechanism of blood coagulation

Blood is coagulated by the hydrolysis of the soluble plasma protein fibrinogen to insoluble fibrin by a serine protease called thrombin. The latter, however, exists only as an inactive proenzyme called prothrombin in the normal circulating blood and has to be activated by another serine protease called factor Xa. Factor Xa has in turn to be produced from another inactive proenzyme, called factor X, during blood coagulation. Activation of this factor X may be carried out by two systems, intrinsic and extrinsic. Both the systems ultimately lead to the activation of factor X to Xa through the stepwise activation of several coagulation-promoting factors, mostly proteases (Table 9.6). Each system

thus forms a *cascade system* in which one molecule of an active factor formed at any step catalyzes the activation of many molecules of another factor at the next step; so, the activation of a few molecules of a factor at the initial step leads finally to the activation of a very large number of molecules of the ultimate factor at the concluding step of the system. The intrinsic system depends on factors occurring in the blood itself while the extrinsic system requires some factors from extravascular tissues (Fig. 9.4).

Intrinsic system of activating factor X:

(a) *Activation of factor XII:* The intrinsic system starts with the activation of a plasma proenzyme called factor XII or *Hageman factor*. This is catalyzed by a protease called *kallikrein* present in small amounts in the plasma normally—kallikrein hydrolyzes factor XII to an active serine protease called factor XIIa. Factor XII, on one hand, becomes more vulnerable to kallikrein-mediated activation on contact with

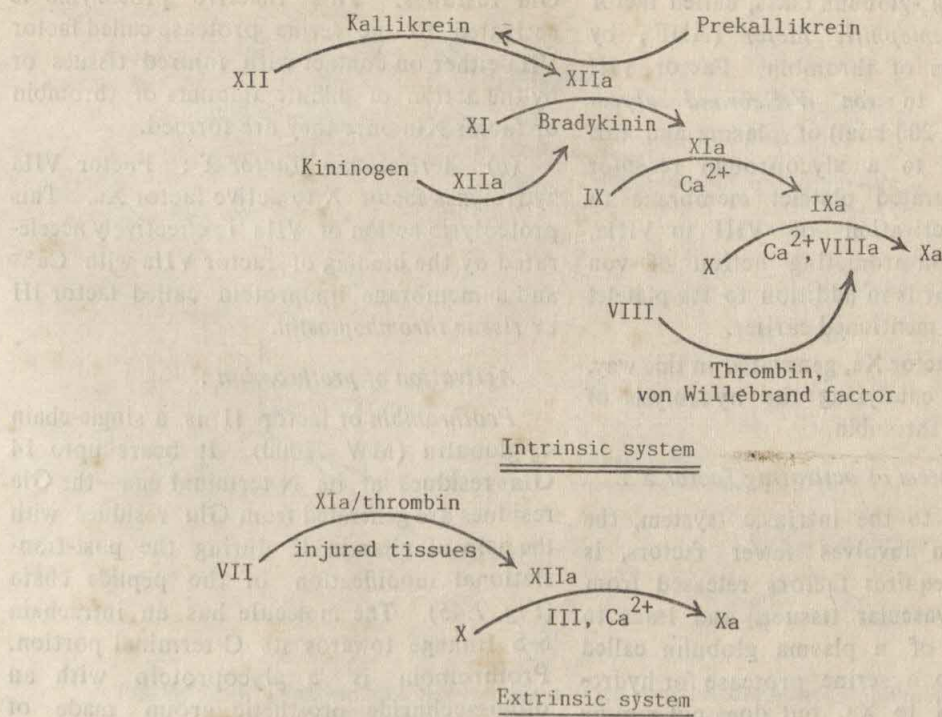


Fig. 9.4. Intrinsic and extrinsic systems for activating factor X.

water-wettable, electronegative and rough surfaces of injured tissues such as exposed collagen fibres and subendothelial basement membranes. On the other hand, as soon as some XIIa has been formed, it catalyzes the hydrolysis of the plasma proenzyme *prekallikrein* into active kallikrein which in turn activates many more factor XII molecules to XIIa. Thus, many XIIa molecules are generated by a rapid autocatalytic effect of factor XIIa through kallikrein.

(b) *Activation of factor XI*: Factor XIIa hydrolyzes a serum globulin called factor XI or *plasma thromboplastin antecedent* (PTA) into an active serine protease called factor XIa. This is accentuated by the nonapeptide called *bradykinin* which is also produced by the XIIa-catalyzed proteolysis of a high-MW *kininogen* of the plasma. A platelet factor may also activate XI to XIa.

(c) *Activation of factor IX*: In presence of Ca^{2+} , factor XIa hydrolyzes a thermolabile β_2 -globulin called factor IX or *Christmas factor* into another serine protease called factor IXa.

(d) *Activation of factor X*: Factor X or *Stuart factor* is a plasma α -globulin carrying a number of Gla residues (see page 177). In presence of disintegrating platelets and Ca^{2+} , it gets bound by its Gla residues to anionic phospholipids of the disrupted platelet membrane through the mediation of Ca^{2+} . This leads to the hydrolysis of an Arg-Ile peptide bond in factor X by factor IXa, releasing another serine protease called factor Xa. However, this activation is slow unless active factor VIIIa has been generated to function as a nonenzymatic receptor for binding factor IXa. Factor VIIIa is formed by the proteolysis of an inactive plasma

glycoprotein of β_2 -globulin class, called factor VIII or *antihemophilic factor* (AHF), by minute amounts of thrombin. Factor VIII remains bound to *von Willebrand glycoprotein* (MW > 200 kdal) of plasma and this complex binds to a glycoprotein receptor on the disintegrated platelet membrane to promote the activation of VIII to VIIIa. This coagulation-promoting action of von Willebrand factor is in addition to its platelet adherence effect mentioned earlier.

The active factor Xa, generated in this way, participates in catalyzing the hydrolysis of prothrombin to thrombin.

Extrinsic system of activating factor X :

In contrast to the intrinsic system, the extrinsic system involves fewer factors, is much faster, requires factors released from damaged extravascular tissues, and leads to the activation of a plasma globulin called factor VII into a serine protease for hydrolyzing factor X to Xa, but does not require the mediation of factors VIII, IX and XII.

(a) *Activation of factor VII*: Factor VII or *proconvertin* is a glycoprotein bearing many

Gla residues. This inactive proenzyme is activated to the serine protease called factor VIIa either on contact with injured tissues or by the action of minute amounts of thrombin or factor XIa once they are formed.

(b) *Activation of factor X*: Factor VIIa hydrolyzes factor X to active factor Xa. This proteolytic action of VIIa is effectively accelerated by the binding of factor VIIa with Ca^{2+} and a membrane lipoprotein called factor III or *tissue thromboplastin*.

Activation of prothrombin :

Prothrombin or factor II is a single-chain α_2 -globulin (MW 72000). It bears upto 14 Gla residues at its N-terminal end—the Gla residues are generated from Glu residues with the help of vitamin K during the post-translational modification of the peptide chain (Fig. 7.45). The molecule has an intrachain S-S linkage towards its C-terminal portion. Prothrombin is a glycoprotein with an oligosaccharide prosthetic group made of hexoses and hexosamines.

Prothrombin is an inactive proenzyme. Its activation requires Ca^{2+} , factors Xa and Va, and the exposed inner side of the membrane

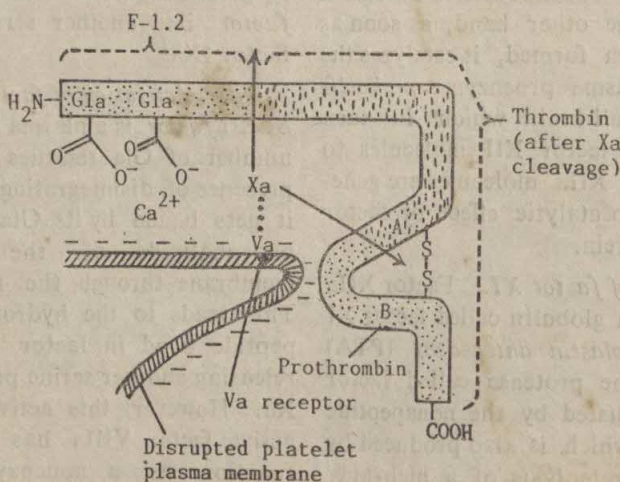


Fig. 9.5. Binding of factors Va, Xa, prothrombin and Ca^{2+} to the platelet membrane and sites of Xa-mediated cleavage of prothrombin.

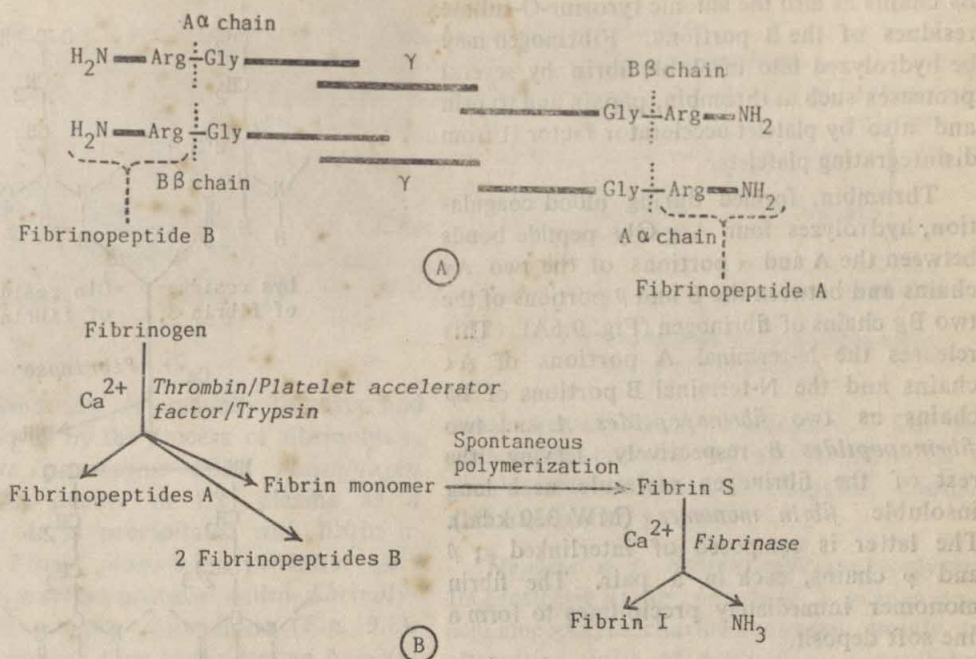


Fig. 9.6. (A) Diagrammatic representation of the structure of fibrinogen molecule.
(B) Conversion of fibrinogen to fibrin polymers.

of disintegrated blood platelets. On the disruption of the platelet membrane, prothrombin molecules are concentrated on the inner side of the membrane by getting bound to the anionic membrane phospholipids through the mediation of Ca^{2+} ions which bridge the phospholipid anionic groups with the anionic Glu residues of prothrombin (Fig. 9.5). Simultaneously, a thermolabile globulin called factor V or *proaccelerin* gets activated to factor Va (accelerin) by minute amounts of thrombin. Factor Va then binds to specific receptors on the exposed inner side of the disintegrated platelet membrane and acts there as a receptor for binding factor Xa molecules formed by either intrinsic or extrinsic system. This locally concentrates factor Xa manifold on the membrane phospholipids to enhance its proteolytic activity. The membrane-bound factor Xa now acts as *prothrombinase*—it binds with

the N-terminal half (F-1.2 region) of the membrane-bound prothrombin and hydrolyzes the latter at two sites shown in Fig. 9.5. Consequently, the N-terminal F-1.2 region of prothrombin is released as an inactive peptide while the C-terminal part of prothrombin is changed to *thrombin* by getting cleaved into A and B chains still held together by the S-S linkage of the molecule. Thus, thrombin (MW 34000) is made of two interlinked peptide chains and is a serine protease.

Conversion of fibrinogen to fibrin :

Fibrinogen (factor I) is a large (46 nm $\times$ 9 nm) fibrous glycoprotein of plasma. It is made of pairs of A α , B β and γ peptide chains interlinked by S-S linkages (Fig. 9.6). Fibrinogen molecules are kept in solution in the plasma by electrostatic repulsion between anionic Glu and Asp residues of the A portions of A α chains and the B portions of

B β chains as also the anionic tyrosine-O-sulfate residues of the B portions. Fibrinogen may be hydrolyzed into insoluble fibrin by several proteases such as thrombin, papain and trypsin and also by platelet accelerator factor II from disintegrating platelets.

Thrombin, formed during blood coagulation, hydrolyzes four Arg-Gly peptide bonds between the A and α portions of the two A α chains and between the B and β portions of the two B β chains of fibrinogen (Fig. 9.6A). This releases the N-terminal A portions of A α chains and the N-terminal B portions of B β chains as two *fibrinopeptides A* and two *fibrinopeptides B* respectively, leaving the rest of the fibrinogen molecule as a long insoluble *fibrin monomer* (MW 330 kdal). The latter is composed of interlinked α , β and γ chains, each in a pair. The fibrin monomer immediately precipitates to form a fine soft deposit.

Formation of fibrin polymers :

With the removal of negatively charged fibrinopeptides from the molecule, fibrin monomers now get spontaneously associated by hydrophobic and hydrogen bonds to form *fibrin S* polymers. These form a relatively soluble soft clot at the site of injury and consist of regularly staggered arrays of fibrin monomers.

Fibrin S is next polymerized into larger and more insoluble *fibrin I* polymer by a transglutaminase called *fibrinase* or factor XIIIa (Fig. 9.6). The latter is produced from *fibrin stabilizing factors* (factors XIII) of plasma and platelets by the hydrolysis of specific Arg-Gly peptide bonds in them under the catalytic action of thrombin. Fibrinase, in presence of Ca^{2+} , catalyzes the formation of an isopeptide bond between the γ -carbonyl group of a glutamine residue of each fibrin S and the ϵ -amino group of a lysine residue of another fibrin S, releasing the amide group of the glutamine residue as

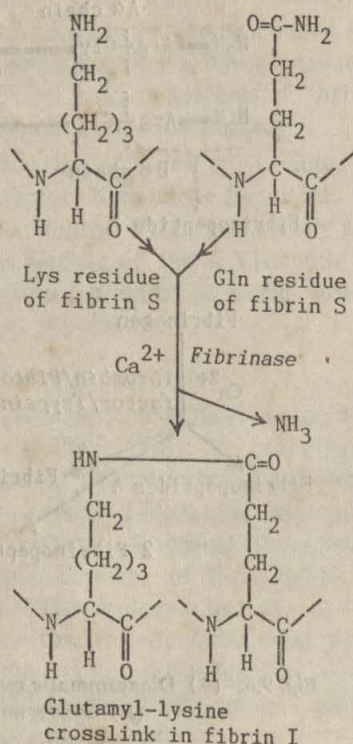


Fig. 9.7. Cross-linking of fibrin S molecules by fibrinase action to form fibrin I.

NH₃ (Fig. 9.7). The fibrin S molecules are thus interlinked to form fibrin I.

Clot retraction :

Platelets and blood cells get entangled in fibrin I fibres to form a firm blood clot. Subsequently, a contractile protein *thrombosin*, released from fused platelets, contracts to cause contraction or retraction of the clot, squeezing out the serum.

Role of calcium in blood coagulation :

Ca^{2+} is essential for blood coagulation. Coagulation of a blood sample is prevented or delayed on addition of oxalates or fluorides which precipitate Ca^{2+} , or of chelating agents like EDTA which form nondissociable complexes with Ca^{2+} . A major role of Ca^{2+} consists of its mediation in bridging the

anionic $\gamma\text{-COO}^-$ group of Glu residues of several coagulation factors such as factors VII, X and prothrombin to the anionic phospholipids of disintegrated platelet membrane. This concentrates such factors on the membrane and enhances their activations and/or actions. Ca^{2+} is also required for the action of fibrinase (factor XIIIa). Ca^{2+} influx into platelets promotes their aggregation and the release of the contents of their granules.

Fibrinolysis

The blood clot is gradually dissolved and finally removed by the process of fibrinolysis. An inactive proenzyme called *plasminogen* (MW 90000) occurs in the plasma as a β -globulin. It is precipitated with fibrin in the clot. *Tissue plasminogen activator* gets activated to a serine protease called *fibrinolysokinase* on contact with fibrin (Fig. 9.8). Fibrinolysokinase then hydrolyzes an Arg-Val peptide bond in plasminogen to form another active serine protease called *plasmin* or *fibrinolysin*. Plasmin then hydrolyzes peptide bonds associated with Arg or Lys residues in fibrin polymers to change them to soluble peptides. Plasmin can also hydrolyze similar peptide bonds in fibrinogen, factor VIII and factor V.

Anticoagulants

Three antithrombins occur in the normal circulating plasma and help in preventing intravascular coagulation in normal uninjured vessels.

Antithrombin III (MW 62000) is a serum globulin which is responsible for about $\frac{3}{4}$ ths of the total antithrombin activity in the normal plasma. It binds with the active sites of serine proteases including thrombin and the thrombin-generating factors VIIa, IXa, Xa, XIa and XIIa. It consequently inactivates those enzymes and thereby prevents both the activation and the action of thrombin. It

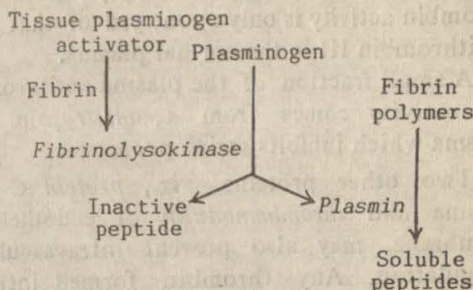


Fig. 9.8. Activation of plasminogen and fibrinolysis by plasmin.

thus prevents blood coagulation. But the anticoagulant action of antithrombin III is considerably enhanced by heparin, another anticoagulant of plasma.

Heparin is a water-soluble proteoglycan. Its prosthetic group consists of a polyanionic acid mucopolysaccharide composed mainly of alternating units of sulfated or nonsulfated iduronic acid and sulfated or N-acetylated glucosamine (Fig. 1.28). It is synthesized and stored in the metachromatic granules of mast cells in lungs, liver, arteries, capillaries and areolar tissues. Basophil leucocytes are also rich in heparin. Heparin is released into the plasma from mast cells and basophils. By virtue of its anionic groups, heparin binds with a specific cationic site of antithrombin III to activate the latter—binding with heparin changes the conformation of antithrombin III to enhance its binding with the serine proteases. Thus, heparin promotes the anticoagulant activity of antithrombin III. Besides, heparin may prevent those steps of the intrinsic system which require the contact of blood with rough, water-wettable, electronegative surfaces, because heparin forms a lining over the inner surface of the vascular endothelium.

An α_2 -globulin, called α_2 plasmin inhibitor, acts as a second antithrombin in the plasma by binding irreversibly with thrombin and

thereby inhibiting it. However, its antithrombin activity is only about $\frac{1}{3}$ rd of that of antithrombin III in the normal plasma.

A small fraction of the plasma antithrombin activity comes from α_1 -antitrypsin of plasma which inhibits serine proteases.

Two other proteins, viz., *protein C* of plasma and *thrombomodulin* of endothelial membrane, may also prevent intravascular coagulation. Any thrombin formed intravascularly binds with thrombomodulin; this complex inactivates factors VIIIa and Va to inhibit respectively the activation of factor X and the binding of Xa to platelet membrane.

Both antithrombin III and α_2 plasmin inhibitor can also bind with and inhibit plasmin, another serine protease, and consequently delay clot dissolution.

Antivitamins for vitamin K, e.g., dicumarol, phenindione and warfarin, have been used clinically as anticoagulants, because they inhibit the vitamin K-mediated conversion of Glu residues to Gla residues in several coagulation factors including prothrombin and factors VII and X, and thereby block both intrinsic and extrinsic systems as well as the action of thrombin (vide page 177).

Methods of delaying or hastening blood coagulation

Coagulation of a blood sample may be prevented or delayed in the following ways: (i) Addition of oxalates or fluorides to precipitate Ca^{2+} . (ii) Addition of chelating agents like EDTA to bind Ca^{2+} into non-dissociable complexes. (iii) Addition of heparin to promote antithrombin III activity. (iv) Preservation of blood in cold to depress the actions of serine proteases for coagulation. (v) Drawal and preservation of blood in silicone-coated containers to prevent damage to platelets.

Blood coagulation may be hastened in the following ways: (i) Alternate freezing and thawing or vigorous shaking of the blood

sample to damage platelets. (ii) Warming the blood sample to enhance protease activities. (iii) Addition of thrombin or platelet extracts to the blood sample. (iv) Contact with rough water-wettable surfaces to damage the platelets.

Defibrination is done by stirring blood with a glass rod so that fibrin threads stick to the stirrer and get separated from blood.

Abnormalities of coagulation

1. *Hypoprothrombinemia*: This is an abnormal lowering of blood prothrombin level due to either an inherited defect in prothrombin synthesis or an extensive hepatic damage or more frequently, a deficiency of vitamin K. Prolonged clotting time and hemorrhagic syndromes result. (See page 177).

2. *Thrombocytopenia*: This is a fall in the blood platelet count. It results in a deficiency of platelet factors necessary for clotting, clot retraction and local vasoconstriction. Delay in coagulation, prolonged bleedings and subcutaneous bleeding spots (*purpura*) are produced.

3. *Hemophilia A*: It is a bleeding disease due to an inborn failure of factor VIII (AHF). The defect involves an X-chromosome-linked Mendelian recessive gene and is transmitted by the female progenies but manifested mainly in male offsprings. Coagulation is abnormally delayed—a soft unstable clot may form only after prolonged bleeding. But platelet adherence to the injured site remains normal.

4. *von Willebrand's bleeding disease*: This is characterized by a reduced adherence of platelets at the injured site and a destabilization of factor VIII, both defects being jointly inherited by an autosomal dominant gene. The defect seems to be an inborn error in the synthesis of a specific oligosaccharide in the prosthetic group of von Willebrand factor.

5. *Christmas disease or hemophilia B*: This bleeding disease occurs mainly in the male and is caused by an inborn deficiency of factor IX due to a defective sex-linked recessive gene.

6. *Hemophilia C*: Here, an inherited deficiency of factor XI may cause a moderate prolongation of clotting time and mild bleedings in both sexes.

7. *Hageman trait*: Congenital deficiency of Hageman factor leads to this bleeding disease with prolonged clotting time.

8. *Parahemophilia*: This bleeding disease is caused by an inherited deficiency of factor V.

9. *Afibrinogenemia*: This disease is characterized by the absence or a low level of fibrinogen in the blood and a consequent inability or delay in blood coagulation. Uncontrollable hemorrhages may result. The disease is caused by a genetic defect in fibrinogen synthesis. The defect is inherited as a non-X-linked recessive trait.

10. *Fletcher trait*: This disease, characterized by a prolonged clotting time and bleedings, results from an inborn deficiency of prekallikrein.

11. *Fitzgerald trait*: This is caused by a genetic defect in synthesizing high-MW kininogen. The clotting time is prolonged and bleedings occur.

12. *Antithrombin III deficiency*: A genetic defect in the synthesis of antithrombin III causes the spontaneous formation of many and widespread blood clots without any injury.

9.8 ANTIBODIES

Antigens

Antigens are complex macromolecules (MW 10000 or higher) of proteins, polysaccharides, glycosphingolipids and synthetic polymers whose entry into an organism, not containing them naturally, evokes the synthesis

and release of specific immunoglobulins called *antibodies*; moreover, an antigen also combines with the resulting antibody to form inactive antigen-antibody complexes. *Immunopotency* of an antigen is its ability to stimulate immunoglobulin synthesis. Antibody or immunoglobulin molecules bear antigen-binding sites which bind with specific restricted segments of the corresponding antigen molecules to form the biologically inactive antigen-antibody complexes. The antibody-binding segments of antigen molecules are called *epitopes* or *antigenic determinants*; they usually occupy the most exposed parts of the antigen molecule like the terminal residues of its sidechains, and bear charged hydrophilic groups. The antigenic determinant is formed of several specific amino acid or hexose residues having specific optical configuration and occurring in specific sequences. There are indications that the antigenic determinant has the size of a pentapeptide or a hexasaccharide in a linear antigen molecule, but may accommodate even more than 15 amino acids in a globular antigen. The primary structure as also the three-dimensional stereo-configuration, maintained by the tertiary structure of the antigenic molecule, determines the specificity of the antigenic determinant for an antibody. On denaturation, the antigen neither evokes antibody formation nor binds with the antibody. An antigen molecule often has more than one antigenic determinant; such a *multivalent antigen* may bind with more than one antibody molecule. The antigen-antibody binding, like most enzyme-substrate combinations, is due to non-covalent bonds like hydrogen, hydrophobic and ionic bonds as also Van der Waals forces. Increase in size and complexity of an antigen molecule enhances its antigenic capacity—a protein with high MW and aromatic amino acid sidechains, has a higher antigenic effect than a small protein or one deficient in aromatic amino acids. In contrast to antigens, *haptens* are small molecules or chemical groups such as

m-aminobenzene sulfonate and dinitrophenyl, which cannot themselves evoke antibody formation, but possess small chemical groups by which they can bind to specific preformed antibodies. However, carrier-hapten conjugates, formed by the covalent coupling of a hapten to a carrier protein, can evoke the formation of anti-hapten antibodies and have been widely used for investigating the antibody-antigen reactions.

Classification of immunoglobulins

Immunoglobulins are γ -globulins acting as antibodies. Normally, they range from 800 mg to 1.8 g in 100 ml plasma and are mixtures of large glycoprotein molecules (MW 150-900 kdal) with isoelectric pHs of 6.3 to 7.3. Some immunoglobulins exist as monomers, each of which is formed of four peptide chains joined by S-S linkages; but others exist as dimers or pentamers of the tetrapeptide monomeric units. Immunoglobulins are classified into five main classes (Table 9.7).

1. *IgA*: Immunoglobulins of this class exist as either monomers or dimers. They are present in the plasma and are secreted in saliva, intestinal juice, tear, urinogenital, nasal and bronchial secretions as well as colostrum, but cannot pass through the placental membrane from the maternal to the fetal blood. IgA may be absorbed from colostrum by human and ungulate newborns without any

digestion, and contributes passive immunity to them.

IgA binds strongly with viral antigens, but less strongly with bacterial antigens. IgA may also participate in the activation of complements (see below) by an 'alternative' pathway, different from the classical pathway of complements; this may be effective in the defence against coliform bacteria in particular. Its presence in sero-mucous secretions like saliva, tear and bronchial secretions protects exposed body surfaces against viral and bacterial infections. IgA may bind on the cell surface of the microbes to coat them; this prevents their adherence to the exposed mucosal surfaces.

According to the type of heavy (H) peptide chains in the molecule, IgA may be divided into two subclasses, viz., IgA₁ and IgA₂. These constitute respectively 80-90% and 10-20% of the total serum IgA.

2. *IgD*: The short-lived immunoglobulins of this class are present in plasma in small amounts only. They exist as tetrapeptide monomers. They are mostly located on the plasma membrane of some lymphocytes, probably to serve as antigen receptors—antigens bound by them may activate or suppress the proliferation of the lymphocytes and immunoglobulin synthesis by them.

3. *IgE*: Monomeric immunoglobulins of this class circulate in plasma in very small

TABLE 9.7. *Immunoglobulins of human plasma.*

	IgA	IgD	IgE	IgG	IgM
Normal range (mg ml ⁻¹)	1-4.5	0.02-0.03	0.0004	7-15	0.4-2.1
% total Ig	12	1	0.001	80	7
MW (kdal)	160,400	185	190	150	900
Sedimentation (S)	7,9	7	8	7	19
Carbohydrate (%)	8	13	12	3	12
Number of tetrapeptide monomers	1,2	1	1	1	5
H chains	α_1, α_2	δ	ϵ	$\gamma_1, \gamma_2, \gamma_3, \gamma_4$	μ_1, μ_2
L chains	κ, λ	κ, λ	κ, λ	κ, λ	κ, λ

amounts normally, but are elevated in blood in case of parasitic infections. IgE mainly participates in tissue sensitization, inflammation and allergy, helping thereby to protect external surfaces of mucous membranes like those of respiratory and intestinal tracts. IgE remains bound with the plasma membrane of mast cells; subsequent binding of an antigen with this membrane-bound IgE stimulates the mast cells to release histamine, platelet activating factor, neutrophil chemotactic factor, eosinophil chemotactic factor, thromboxane A_2 and other anaphylactic agents. Actions of these factors in turn bring about inflammations, allergies and ejection of helminthic parasites from the intestinal wall.

4. *IgG*: Monomeric immunoglobulins of this class form about 80% of the circulating immunoglobulins in normal plasma and possess smallest molecules among the immunoglobulins. So, IgG diffuses more readily than other immunoglobulins into extravascular fluids and is the most abundant immunoglobulin in those body fluids also. According to the type of H chains in the molecule, IgG is divided into four subclasses, viz., IgG_1 , IgG_2 , IgG_3 and IgG_4 . In the normal serum, these four IgG subclasses constitute respectively 65%, 23%, 8% and 4% of the total IgG.

Permeability of placental membranes to IgG allows the maternal IgG to pass into the fetus, enhancing its immunity before and immediately after birth. IgG_2 crosses the placental membranes far less than the other IgG subclasses. IgG binds with both bacterial and viral antigens, but less strongly than the binding of those antigens with IgM and IgA respectively. Nevertheless, its high serum concentration, high diffusibility to extravascular fluids and ability to cross the placenta make IgG highly important in body defence. IgG is the principal antibody involved in *antitoxic activities*. It is also of major importance in causing *opsonic adherence* of antigenic particles to the membrane of phago-

cytes, leading to the phagocytosis of those particles—IgG molecules bind to the surface of antigenic particles on one hand and to specific receptors on the phagocyte membrane on the other, causing the antigenic particles to be anchored on the phagocyte surface. In a similar way, IgG binds antigenic particles like bacteria on the surface of *natural killer* (NK) cells, leading to the extracellular destruction of such particles without phagocytosis. IgG, bound to the antigenic cell surface, binds and activates complements of plasma through the classical pathway, leading to the lysis of the antigenic cell. IgG_3 , IgG_1 , IgG_2 and IgG_4 help in *complement fixation* as well as opsonic adherence in the descending order.

IgG molecules may also bind to specific receptors on the membrane of platelets, leading to the aggregation of platelets and the release of vasoactive amines like serotonin from them.

5. *IgM*: Immunoglobulins of this class have the highest MW among immunoglobulins. They cannot pass through the placenta, cannot diffuse much into the extravascular body fluids, and remain confined mainly to the blood. Circulating IgM molecules are pentamers, each composed of five tetrapeptide monomeric units. According to the types of H chains in their molecules, IgM antibodies may be divided into two subclasses, IgM_1 and IgM_2 .

IgM can bind strongly with bacterial antigens, but far less strongly with viral antigens. Although IgM antibodies are far lower in concentration than IgG antibodies in normal blood, the former represent the major class of antibodies produced early in the immune response to infection. On getting bound to the antigenic cell membrane, IgM antibodies crosslink many antigenic cells to cause their *agglutination* or clumping. Besides, like the IgG antibodies, IgM antibodies bound by antigenic cells can also bind complements, activating them through the classical pathway and consequently leading to the *cytolysis* of the bacterial cell. IgM

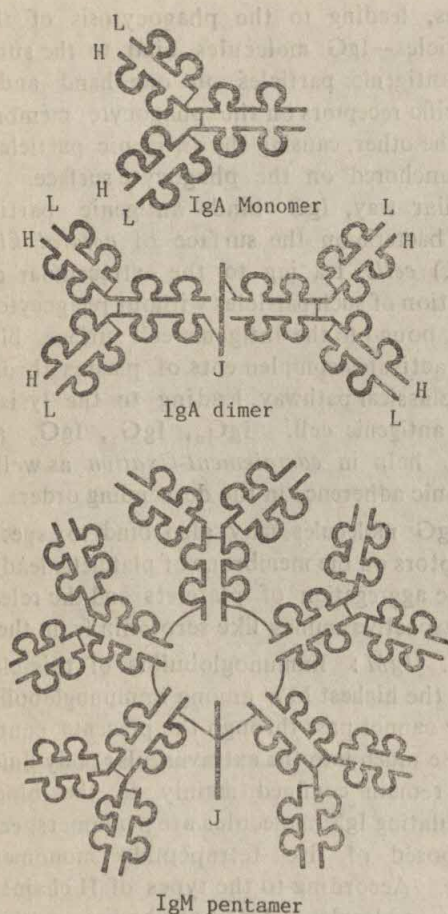


Fig. 9.9. Schematic diagram of different forms of immunoglobulins. Thin lines represent S-S linkages.

possesses far higher ability for this complement fixation than IgG.

Monomeric IgM molecules remain anchored to the plasma membrane of B lymphocytes to serve as receptors for specific antigens—on the binding of the latter with the membrane-bound IgM, proliferation and differentiation of B lymphocytes get triggered.

Structure of immunoglobulins

An immunoglobulin molecule is often an Y-shaped monomer, formed of two light L chains (MW 23 kdal) and two heavy H chains

(MW 50-75 kdal), joined by disulfide linkages (Fig. 9.9). Some of the immunoglobulins, however, are oligomers of 2-5 such tetrapeptide monomeric units, interlinked by either a small J peptide chain (MW 15 kdal) or a disulfide (S—S) linkage. IgG, IgD and IgE exist as monomers, IgM as a pentamer, and IgA as either a monomer or a dimer. There are two classes of L chains, viz., κ and λ . But there are five major classes of H chains, viz., α , δ , ϵ , γ and μ ; α class of H chains has two subclasses, α_1 and α_2 ; γ class consists of γ_1 , γ_2 , γ_3 and γ_4 subclasses; μ class has two subclasses, μ_1 and μ_2 .

The tetrapeptide monomer carries two peptides belonging to a specific type of L chain and two peptides of a particular type of H chain (Fig. 9.10). Each L chain is joined by an S-S linkage with an H chain while the two H chains are bound together by one to five S-S linkages. The N-terminal end of each peptide chain is situated towards the divergent ends of the arms of Y while the C-terminal end is directed towards the root of the stem of Y.

The secondary structure of an L or H chain consists mainly of α -helix. However, at the junction of the divergent arms of the Y-shaped monomer with its stem, each H chain has a nonhelical segment containing several proline residues which prevent α -helix formation. This part of the H chain is consequently flexible and is called its *hinge*, because this allows the divergent arms of the Y-shaped monomer to swing apart from one another even upto 180° so as to accommodate antigens of diverse sizes between the arms.

The three-dimensional tertiary structure of an L or H chain consists of several globular domains or regions. Each domain is a looped segment of the peptide chain and is formed by an intrachain S-S linkage between two cysteine residues at different locations on the same peptide chain (Fig. 9.10). Towards the N-terminal end of each L chain, there is a variable domain (VL) in which the amino

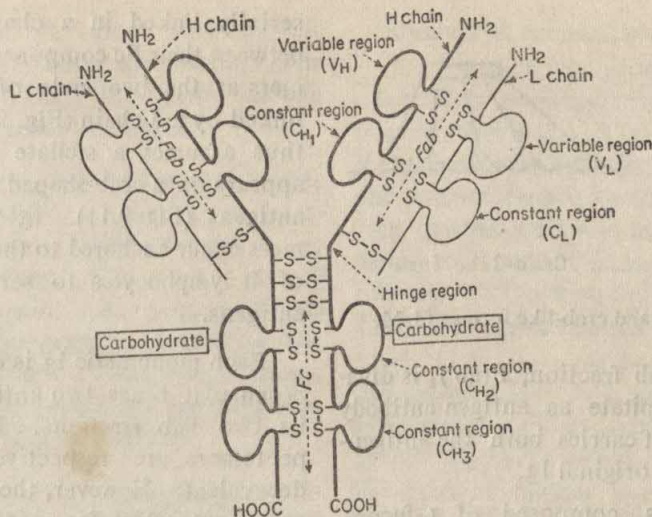


Fig. 9.10. Structure of monomeric immunoglobulin.

acid sequence varies from molecule to molecule even among the L chains of a particular type. The V_L is followed on its C-terminal side by a *constant domain* (C_L) with an identical amino acid sequence in all the L chains of a specific type. Each H chain has one variable domain (V_H) near its N-terminal end and in most cases, three constant domains (CH_1 , CH_2 and CH_3) towards its C-terminal end. The CH_1 domain is located on that segment of the H chain which forms the divergent arm; the other constant domains starting from CH_2 are located on that segment of the H chain which forms the stem of Y. In each H chain of IgM molecules, the hinge region is replaced by a nonflexible extra domain which becomes the CH_2 domain of IgM—consequently, the domains corresponding to CH_2 and CH_3 of IgG become CH_3 and CH_4 domains, respectively, in IgM.

A monomeric immunoglobulin possesses two *antigen-binding sites*, each of which is formed by the interaction between the V_L and V_H domains of one of the divergent arms of the Y-shaped molecule. Each variable domain contains several *hypervariable regions*; these

show high incidences of variations in amino acids among the molecules of even the same type of chain and determine the specificity of the antigen-binding site. Compared to the bivalency of a monomeric Ig for antigen binding, oligomeric Ig molecules have higher valencies, e.g., pentameric IgM has a maximum antigen-binding valency of 10. The CH_2 domain of IgG possesses *binding sites for complements*. The CH_3 domain of IgG may bind the immunoglobulin to the surfaces of monocytes, macrophages and polymorphs.

Papain hydrolyzes an immunoglobulin monomer into an *Fc* and two identical *Fab* fractions (Fig. 9.10). *Fc* is crystallizable; it consists of the C-terminal halves of two H chains joined by S-S linkages, and cannot bind antigens due to the absence of antigen-binding sites. *Fab* consists of the N-terminal half of an H chain, united by an S-S linkage with the corresponding L chain. *Fab* can bind the specific antigen as it still carries its V_L and V_H domains constituting an antigen-binding site; but *Fab* cannot precipitate the antigen. Pepsin hydrolyzes Ig to give an $F(ab')_2$ fraction having two L chains joined with interlinked N-terminal halves of respective H chains; unlike

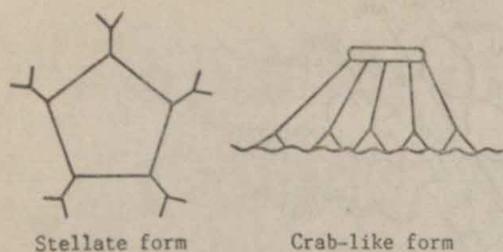


Fig. 9.11. Stellate and crab-like forms of IgM.

the monovalent Fab fraction, $F(ab')_2$ is divalent and can precipitate as antigen-antibody complexes because it carries both the antigen-binding sites of the original Ig.

Oligosaccharides, composed of L-fucose, D-mannose, D-galactose, N-acetyl-D-glucosamine and sialic acids, remain covalently bound to the CH regions by N-glycosidic linkages.

Irrespective of the class, immunoglobulins carry pairs of either κ or λ type of L chains, the κ type appearing with twice the frequency of the λ type. But the type of H chain differs from class to class—IgA, IgD, IgE, IgG and IgM carry pairs of α , δ , ϵ , γ and μ chains respectively. Moreover, each class of immunoglobulin is further subdivided into subclasses according to the subtypes of their H chains; e.g., IgA₁ and IgA₂; IgM₁ and IgM₂; IgG₁, IgG₂, IgG₃ and IgG₄.

IgA occurs in seromucous secretions as a dimer. The latter is formed in plasma cells by linking two tetrapeptide monomers by a cysteine-rich peptide called the J chain (MW 15 kdal). The dimer released by plasma cells is taken up by secretory epithelial cells, bound to a peptide called the secretory component (SC) of MW 60 kdal, and secreted in seromucous secretions—binding with SC protects the Ig dimer against proteolysis and probably also helps in its transport across the epithelial cell into the secretory lumen.

IgM occurs mostly as a pentamer. In this pentamer, five tetrapeptide monomers are

serially linked in a chain by S-S linkages between their Fc components while the monomers at the two ends of the chain are inter-linked by a J chain (Fig. 9.9). The pentamer thus assumes a stellate form in solution but appears in a crab-shaped form when bound to antigens (Fig. 9.11). IgM tetrapeptide monomers occur anchored to the plasma membrane of B lymphocytes to serve as receptors for antigens.

Each monomeric Ig is divalent for antigens because it bears two antigen-binding sites on its two Fab fractions. IgA dimers and IgM pentamers are respectively tetravalent and decavalent. However, the IgM pentamer is at most pentavalent for macromolecular antigens because the inflexibility of the IgM molecule as well as the large size of already bound antigens prevents the access of more antigens to the remaining antigen-binding sites (*steric hindrance*).

Lymphocytes and body immunity

Circulating small lymphocytes are of two major types: (a) *T lymphocytes* or graft-rejection cells participate in the *cell-mediated immunity* against viruses and fungi, help to reject foreign tissue transplants, destroy tumors, and enhance macrophage activity by releasing large *lymphokine* molecules; (b) *B lymphocytes* synthesize immunoglobulins and differentiate into *plasma cells* which protect the body against viral reinfections and bacteria, by secreting immunoglobulins in blood (*humoral immunity*).

Primitive lymphoid stem cells proliferate in the fetal bone marrow. Some of these cells reach the fetal thymus and continue differentiating under the action of the thymic hormone, thymosin, to produce T lymphocytes. The latter are then transported by blood to the periarteriolar white pulp of spleen and the paracortical lymphoid tissue of lymph nodes; there the cells mature for some time and

ultimately pass into the blood through the lymph. In lymphoid tissues and blood, some T cells are transformed into large mobile *lymphoblasts* in presence of antigens.

In birds, some proliferating lymphoid cells pass from the fetal bone marrow to an intestinal lymphoid pouch called the *bursa of Fabricius* where they differentiate into B lymphocytes; in mammals, the bursa is absent and the B cells differentiate probably in the fetal bone marrow itself. From these tissues, the B cells are transferred to the lymphoid follicles of lymph nodes and spleen.

The differentiation of lymphoid stem cells into B lymphocytes appears to be independent of the presence of antigens and is induced by the microenvironment of the avian bursa and the mammalian fetal bone marrow. According to the *clonal selection model* of immunoglobulin synthesis, each B lymphocyte carries the gene which codes for the synthesis of a specific immunoglobulin. Immunoglobulin molecules, so synthesized, are incorporated on the B cell membrane where they serve as receptors for antigens by virtue of their antigen-binding sites. A particular antigen can bind with only such among the B lymphocytes as bear surface immunoglobulins with specificity for that antigen. As the specific antigens combine with the antigen-binding sites of the surface antibodies of a B cell, the latter repeatedly divides and differentiates to give rise to a clone or large population of daughter B cells capable of synthesizing the same specific immunoglobulin as the mother B cell. Many B cells of this clone develop into plasma cells which actively secrete the specific immunoglobulin into the plasma to combat the antigen; but some continue as small B lymphocytes with the surface-bound antibody and form an expanded population of *memory cells* for a secondary immune response in case of any subsequent exposure to the same antigen.

Clinical abnormalities

1. **Bruton's X-linked agammaglobulinemia**: This *immunodeficiency disease* results from an inborn failure of B cell differentiation. It is transmitted as an X-linked recessive trait and is characterized by the absence of circulating plasma cells and immunoglobulins and a failure of humoral immunity against bacteria, although the cell-mediated immunity against fungi and viruses remains unimpaired.

2. **DiGeorge's syndrome**: In this inborn immunodeficiency disease, T cells fail to differentiate and the cell-mediated immunity against fungi and viruses is seriously impaired, but there is neither any disturbance in the humoral immunity against bacteria nor any fall in blood immunoglobulin level.

3. **Hypergammaglobulinemia**: A malignant proliferation of plasma cells in *multiple myeloma* causes an overproduction and rise in the serum titre of immunoglobulin. Although both L and H chains are produced in excess in the malignant plasma cells, rise in the L chain synthesis may often exceed that of the H chain synthesis, resulting in the urinary excretion of the excess L chains in the form of the so-called *Bence-Jones proteins*. The latter have low MW and unlike normal serum albumin and globulins, coagulate between 40° and 60°C, but redissolve on boiling. Each Bence-Jones protein molecule is formed of two identical and interlinked L peptide chains. This abnormal protein differs from patient to patient, but is of a single type in any particular patient, because the malignancy involves a single type of IgG-producing cell and causes overproduction and high serum titre of a single molecular species of immunoglobulin. Such genetic defects with abnormal overproduction of a single type of Ig are called *monoclonal gammopathies*.

Antigen-antibody reactions

Antibodies or immunoglobulins bind specifically with the antigens which have evoked

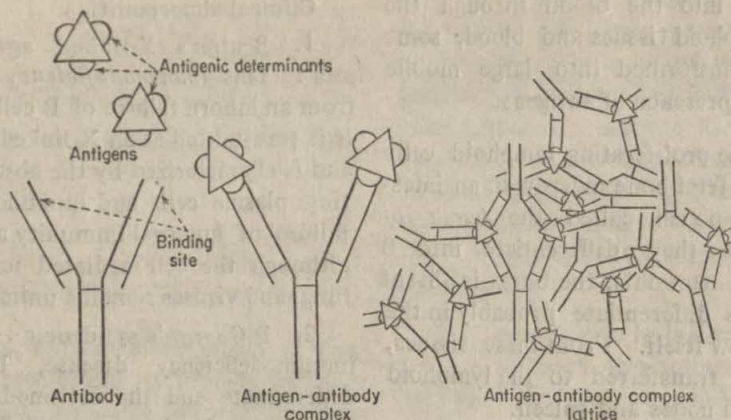


Fig. 9.12. Antigen-antibody reaction.

their production. Each immunoglobulin monomer is bivalent for antigens as it carries two antigen-binding sites. The specificity for antigens is determined by the amino acid residues in the hypervariable regions of the antigen-binding sites while the flexible hinge region of the antibody facilitates the accommodation of antigens of diverse dimensions. The antigen-binding site of the immunoglobulin binds with the antigenic determinant of the antigen molecule by non-covalent bonds like ionic, hydrophobic and hydrogen bonds.

The antigen-antibody reaction occurs in two stages. Initially, the noncovalent binding of the antigen and the antibody produces small and soluble antigen-antibody complexes (*primary union*). Subsequently, these complexes change their 3-dimensional forms and aggregate into more complex and insoluble crystal lattices with physically demonstrable transformations like precipitation and agglutination (*secondary effects*).

An antigen molecule may possess several antigenic determinants and may consequently bind with the antigen-binding sites of more than one antibody molecule. On the other hand, the bivalent monomeric IgG may bind with two antigen molecules while the decavalent pentameric IgM or the tetravalent

dimeric IgA can bind with many antigen molecules. Thus, a complex three-dimensional *antigen-antibody lattice* may be formed by the cross-linking of many multivalent antigens with the bivalent or multivalent antibody molecules (Fig. 9.12). This may lead to several secondary effects subserving the functions of immunoglobulins.

(a) Cross-linking of many antigenic macromolecules by antibodies results in the *precipitation* of large insoluble aggregates of antigen-antibody complexes; the participant antibodies may be called *precipitins*.

(b) Where the antibodies get bound to the antigenic toxins of microbes, plants and animals and inactivate those toxins, the antibodies are often IgG molecules called *antitoxins*. This *antitoxic effect* may result from either a change in the 3-dimensional conformation of the antibody-bound toxin molecule or a *steric hindrance* against the binding of host molecules with the toxin due to the binding of the antibody close to the substrate-binding site of the toxin.

(c) Sometimes, antibodies bind with the surface antigens of a number of foreign cells or antigen-coated body cells, forming multiple cross-links between those cells so as to overcome their mutual electrostatic repulsions

and to cause their clumping or *agglutination*; such antibodies are widely known as *agglutinins* and include the serum anti-A and anti-B agglutinins of both IgM and IgG classes—evidently, the multivalence of IgM antibodies makes them stronger agglutinins compared to bivalent IgG.

(d) IgG antibodies in particular may function as *opsonins*—they bind by their antigen-binding sites with the surface antigens of foreign cells, and by their Fc parts with Fc-receptor sites on macrophages and neutrophils, causing an *opsonic adherence* of the phagocyte with the antigenic cell and promoting the phagocytosis of the latter.

(e) Similarly, IgG molecules may bind with surface antigens on antigenic cells and also with Fc-receptors on the membrane of *killer or K cells*, anchoring the antigenic cell on the K cell; this helps in the killing and *lysis* of the antigenic cell by cytotoxic and cytolytic chemicals such as *perforin* secreted by the K cell.

(f) IgG and IgM antibodies may function as *lysins* in promoting the rupture or cytolysis of antigenic cells through complement fixation. The antigen-binding sites of these antibodies bind with the surface antigens of the antigenic cell while the complement-binding sites of the Fc parts of those cell-bound antibodies fix some circulating globulins called *complements*; the latter consequently get activated through the classical complement pathway and catalyze the lysis of the cell. IgA also may fix complements by an alternative pathway.

(g) Antigen molecules may bind with and cross-link IgE molecules (*reagins*) bound to the plasma membrane of mast cells and basophils. This triggers the release of anaphylactic substances from those cells, leading to *inflammation* and *anaphylaxis*.

(h) Immunoglobulin molecules such as IgM monomers remain incorporated in the

plasma membrane of B lymphocytes to serve as *antigen receptors*. When specific antigen molecules bind with and cross-link these membrane-bound immunoglobulins, the B lymphocyte is induced to proliferate into a clone of B lymphocytes with similar genes. Some of these B cells differentiate into plasma cells for secreting immunoglobulins which may react with and destroy the inducing antigens. Other B cells of the clone are retained as memory cells for immune response in case of subsequent encounters with the same type of antigen.

Antibodies capable of binding with antigens even at low temperatures like 4°C are called *cold antibodies* while *warm antibodies* participate in antigen-binding particularly at warm temperatures like 37°C. *Complete antibodies* can react with the corresponding antigens even in a saline medium while *incomplete antibodies* require a colloidal medium for this purpose. Serum anti-A and anti-B agglutinins of IgM class are complete and cold antibodies while serum IgG agglutinins are incomplete and warm antibodies.

9.9 REAGINS AND ANAPHYLACTIC SUBSTANCES

Reagins are immunoglobulins, mostly of the IgE class, which remain bound on the plasma membrane of mast cells and basophil leucocytes and mediate in *anaphylaxis* or an *immediate hypersensitivity reaction*. Antigens which induce the synthesis of IgE immunoglobulins and can bind to those IgE molecules, are called *allergens*. Pollens, some foods like strawberries and egg albumin, some antibiotics including penicillin, sulfobromophthalein, some insect venoms like bee and wasp sting venoms, etc., contain allergens for sensitive individuals.

IgE molecules remain bound to specific IgE receptors on the plasma membrane of mast cells and basophils. The IgE receptor is an integral membrane glycoprotein—its

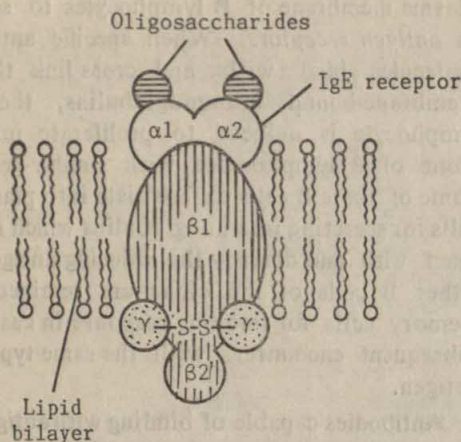


Fig. 9.13. Diagrammatic representation of the structure of an IgE receptor in the mast cell membrane.

α chain bears the oligosaccharide groups and projects from the outer surface of the membrane, the β chain spans the membrane while two γ chains interlinked by an S-S linkage project from the cytoplasmic surface of the membrane (Fig. 9.13). The α chain of the IgE receptor binds with a specific site at the junction of CH_2 and CH_3 domains of the Fc part of IgE. Thus, the IgE molecule remains bound to the mast cell membrane by its Fc part while its divergent Fab arms bearing the antigen-binding sites project from the outer surface of the membrane.

Specific allergens may cross-link the membrane-bound IgE molecules by binding with the antigen-binding sites of several IgE molecules simultaneously. This allergen-mediated cross-linking of membrane-bound IgE molecules triggers the release of anaphylactic agents from the cytoplasmic granules of mast cells and basophils to the surrounding extracellular fluid, causing an extensive degranulation of those cells (Fig. 9.14). Complement fractions C3a, C4a and C5a may also trigger the degranulation of mast cells and basophils, and are accordingly called *anaphylatoxins*. But these substances, produced in the

complement activation pathways, do not act through the IgE receptors.

When the reagins (IgE) on the mast cell membrane are cross-linked by allergens, this activates methyltransferases, phospholipases A_2 and C, and serine esterases; phosphatidylinositol is cleaved to inositol triphosphate and diacylglycerol, cytoplasmic Ca^{2+} concentration rises due to an influx of Ca^{2+} from ECF as well as a translocation of mitochondrial Ca^{2+} to the cytoplasm, and cytoplasmic concentration of cyclic GMP is enhanced. These biochemical events lead to two types of effects. First, the cytoplasmic granules move to the cell margin and their membranes fuse with the plasma membrane to release the granular contents into the ECF; this membrane fusion and consequent degranulation are promoted by membrane-active *fusogens* such as lysophosphatidate produced from phospholipids in the biochemical events. Anaphylactic substances such as histamine, chemotactic factors and platelet activating factor are thus released from the granules where they were so long remaining stored. Secondly, metabolism of arachidonic acid is enhanced in the cell; arachidonate is released from membrane phospholipids by the action of phospholipases A_2 and C, activated by the allergen-mediated cross-linking of membrane-bound IgE. Arachidonate is in turn oxidized to leukotrienes, prostaglandins and thromboxanes. These freshly synthesized substances are also released from mast cells and produce further anaphylactic and inflammatory changes. Anaphylactic agents released by mast cells and basophils include the following.

(i) *Histamine*: This biologically active amine remains stored in the granules of mast cells and basophils, and is released from there during degranulation. It causes dilatation of small blood vessels, increases the permeability of capillaries, contracts the smooth muscles on bronchioles to constrict them and

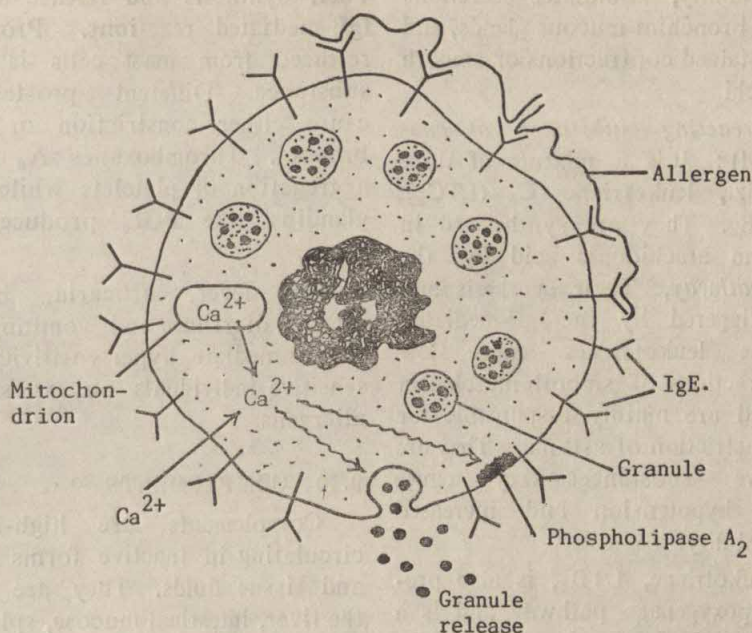


Fig. 9.14. Degranulation of a mast cell following allergen-mediated cross-linking of membrane IgE molecules.

stimulates the secretions of nasal and bronchial mucous glands.

(ii) *Serotonin*: This biologically active amine is also released from the granules of mast cells and platelets. It is a vasodilator substance. It also increases capillary permeability.

(iii) *Neutrophil chemotactic factors of anaphylaxis (NCF-A)*: Released from mast cell granules, they exert positive chemotactic action on neutrophils and thereby promote neutrophil infiltration of the inflamed tissue. These are macromolecular proteins (MW $\approx$ 750 kdal).

(iv) *Eosinophil chemotactic factor of anaphylaxis (ECF-A)*: It is a small acidic tetrapeptide released from mast cell granules. It causes chemotaxis of eosinophil leucocytes to the site of anaphylaxis. Eosinophils, on reaching the site, help to moderate the effects of anaphylactic agents released from mast

cells. For example, histaminase, aryl sulfatase and phospholipase, released from the granules of eosinophils, hydrolyze and inactivate respectively histamine, SRS-A and platelet activating factor from mast cell granules.

(v) *Platelet activating factor (PAF)*: It is probably an acetyl glycerol ether of phosphorylcholine. It is released from mast cell granules. It causes aggregation of platelets, and releases platelet factors from the platelets.

(vi) *Heparin*: This mucoprotein, released from mast cell and basophil granules, is an anticoagulant. But its action in human anaphylaxis is not known.

(vii) *Kinin-generating proteases*: Released from basophil and mast cell granules, they hydrolyze inactive factor XII (Hageman factor of blood coagulation pathway) to active factor XIIa. It activates plasma kininogen to bradykinin as also prekallikrein to active kallikrein. Bradykinin in turn increases

capillary permeability, stimulates secretions from nasal and bronchial mucous glands, and causes slow sustained contractions of smooth muscles of bronchi.

(viii) *Slow-reacting substance of anaphylaxis (SRS-A)*: It is a mixture of three leukotrienes, viz., leukotriene C_4 (LTC_4), LTD_4 and LTE_4 . They are synthesized in mast cells from arachidonic acid by the *lipoxygenase pathway*. Their synthesis and release are triggered by the IgE-mediated reaction. These leukotrienes cause slow sustained contractions of smooth muscles on bronchioles and are mainly responsible for the bronchoconstriction of asthma. They are also vasoactive substances—they cause vasodilatation, hypotension and increased vascular permeability.

Another leukotriene, LTB_4 , is also produced in the lipoxygenase pathway and is a strong chemotactic factor for neutrophils. It also enhances vascular permeability.

(ix) *Thromboxanes and prostaglandins*: These are synthesized from arachidonic acid by the *cyclo-oxygenase pathway* in mast cells.

Their synthesis and release are triggered by IgE-mediated reactions. Prostaglandin D_2 released from mast cells is a vasodilator substance. Different prostaglandins may cause either constriction or dilatation of bronchi. Thromboxanes A_2 and B_2 cause aggregation of platelets while some prostaglandins like PGI_2 produce the opposite effect.

Hay fever, urticaria, bronchospasms, asthma, diarrhoea and vomiting are some of the immediate hypersensitivity reactions in sensitive individuals on exposure to specific allergens.

9.10 COMPLEMENTS

Complements are high-MW globulins circulating in inactive forms in the plasma and tissue fluids. They are synthesized by the liver, intestinal mucosa, spleen and macrophages. Complements are designated as C1, C2, C9, etc. These act in cooperation with IgG and IgM to cause lysis of antigenic cells. They also help in inflammations, anaphylaxis, chemotaxis and phagocytosis.

TABLE 9.8. *Components of the complement system.*

Complement	Nature of globulin	MW (kDa)	Sedimentation coefficient (S)	Serum concn. (mg/dl)
C1q	γ_2	400	11	7
C1r	β	190	7.5	3.4
C1s	α_2	87	4.5	3.1
C2	β_1	117	4.5	2.5
C3	β_1	185	9.5	90-250
C4	β_1	206	10	10-60
C5	β_1	180	8.7	8.5
C6	β_2	128	5.5	7.5
C7	β_2	121	5.5	5.5
C8	γ_1	153	8	5.5
C9	α_2	72	4.5	6
Factor B	β_2	95	5	20
Factor D	α_2	25	3	0.12
Factor H	β_1	150		50
Factor I	β	88		3.4
Properdin	γ_2	224	5.4	2.5

Complements are activated through either a classical pathway or an alternative pathway. The first complement component, activated in either pathway, catalyzes the activation of a larger number of molecules of the next complement of the series; each molecule of the activated second complement in turn catalyzes the activation of a still larger number of molecules of the third complement component of the series. This is repeated at each step constituting a *cascade system* similar to that for blood coagulation. Because the product of each step acts as the catalyst for the next, this cascade system amplifies the response progressively, rapidly and vastly. Several of the activated complements function as serine esterases.

Classical complement pathway

The classical pathway for the activation of complements is induced by antigen-antibody complexes. It may also be induced by some cellular membranes, C-reactive protein or trypsin-like proteases such as plasmin.

Normally, C1q, C1r and C1s remain bound to each other in the plasma by Ca^{2+} -dependent bonds to form a stable trimolecular

complex called C1qrs. C1q possesses a collagen-like helical stem made of 6 subunits, each consisting of 3 polypeptide chains. Each subunit has a triple helix structure which fans out from other subunits and terminates at a free globular end bearing an antibody-binding site. C1q thus possesses 6 antibody-binding sites and is hexavalent for IgG antibodies.

IgM and IgG antibodies have complement-binding sites in the hydrophobic regions of respectively CH_3 and CH_2 domains. After IgM or IgG molecules have bound to the surface antigens on an antigenic cell by their antigen-binding sites, their complement-binding sites get noncovalently bound to the antibody-binding sites of the C1q part of the C1qrs complex. The immunoglobulins consequently serve to link the C1qrs complex to the antigenic cell membrane. This is called *complement fixation* and serves to activate the C1q part of the C1qrs complex to C1q̄. IgM, IgG₃, IgG₁ and IgG₂ possess the complement-fixing ability in the decreasing order; IgG₄ has very little complement-fixing ability.

C1q̄ next nonenzymatically activates C1r into an active serine esterase called C1r̄. The

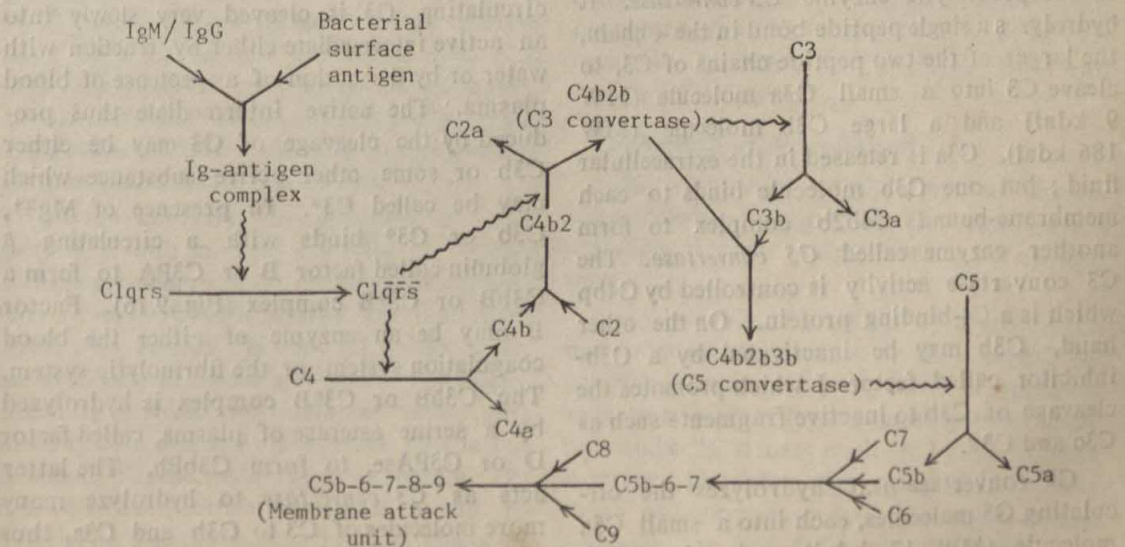


Fig. 9.15. The classical complement pathway.

latter in turn catalyzes the irreversible covalent activation of $C1s$ into another serine esterase called $C1s$ by hydrolyzing $C1s$ in presence of Ca^{2+} . In this way, the $C1qrs$ complex bound to the antigenic cell membrane is activated into the $C1qrs$ complex (Fig. 9.15).

$C1s$ part of the $C1qrs$ complex next hydrolyzes a single peptide bond in the α chain, the largest of the three peptide chains of $C4$, to produce a bigger $C4b$ molecule (MW 197 kdal) made of three chains, and a small $C4a$ peptide molecule (MW 8.7 kdal). $C4b$ then binds either to a new site on the antigenic cell membrane or to the membrane-bound $Ig-C1qrs$ complex. This is followed by the binding of $C2$ to the membrane-bound $C4b$ to form a $C4b2$ complex in presence of Mg^{2+} . $C1s$ now hydrolyzes the $C2$ part of this complex into a small $C2a$ and a large $C2b$ fractions, the former being released in the plasma and the latter remaining bound to $C4b$ as the $C4b2b$ complex. (Earlier, the small and large fractions of $C2$ were called $C2b$ and $C2a$ respectively; but these are now redesignated as $C2a$ and $C2b$ respectively to keep uniformity with the fractions of other complements). $C4b2b$ can function as the proteolytic enzyme *C3 convertase*. It hydrolyzes a single peptide bond in the α chain, the larger of the two peptide chains of $C3$, to cleave $C3$ into a small $C3a$ molecule (MW 9 kdal) and a large $C3b$ molecule (MW 186 kdal). $C3a$ is released in the extracellular fluid; but one $C3b$ molecule binds to each membrane-bound $C4b2b$ complex to form another enzyme called *C5 convertase*. The *C3 convertase* activity is controlled by $C4bp$ which is a $C4$ -binding protein. On the other hand, $C3b$ may be inactivated by a $C3b$ -inhibitor called factor *I* which promotes the cleavage of $C3b$ to inactive fragments such as $C3c$ and $C3d$.

C5 convertase next hydrolyzes the circulating $C5$ molecules, each into a small $C5a$ molecule (MW 17 kdal) and a large $C5b$

molecule (MW 163 kdal). $C5a$ is released in the extracellular fluid, but $C5b$ binds to a site on the antigenic cell membrane. Circulating $C6$ and $C7$ molecules then bind to $C5b$ to form a $C5b-6-7$ complex. The latter binds with a membrane site and then with circulating $C8$ and $C9$ molecules to form a $C5b-6-7-8-9$ complex attached to the antigenic cell membrane. This complex is called the *membrane attack unit* and produces a lesion in the outer lipoprotein membrane overlying the cell wall of Gram-negative bacterial cell, leading to the lysis of the latter.

Besides the final $C5b-6-7-8-9$ membrane attack unit, the intermediate products such as $C3a$, $C5a$, $C3b$ and $C5b-6-7$ have numerous activities which will be discussed below.

Alternative complement pathway

This differs from the classical pathway in the steps for activating $C3$. The alternative pathway does not need IgG or IgM for its activation; its activation is induced by IgA , bacterial polysaccharides, lipopolysaccharides and trypsin-like enzymes such as plasmin.

Even in the absence of any inducing factor, circulating $C3$ is cleaved very slowly into an active intermediate either by reaction with water or by the action of a protease of blood plasma. The active intermediate thus produced by the cleavage of $C3$ may be either $C3b$ or some other active substance which may be called $C3^*$. In presence of Mg^{2+} , $C3b$ or $C3^*$ binds with a circulating β globulin called factor *B* or $C3PA$ to form a $C3bB$ or $C3^*B$ complex (Fig. 9.16). Factor *B* may be an enzyme of either the blood coagulation system or the fibrinolytic system. The $C3bB$ or $C3^*B$ complex is hydrolyzed by a serine esterase of plasma, called factor *D* or $C3PAse$, to form $C3bBb$. The latter acts as *C3 convertase* to hydrolyze many more molecules of $C3$ to $C3b$ and $C3a$, thus enhancing the formation of $C3bBb$ or $C3$

start a membrane lesion, binding of two or more C9 molecules to that complex greatly accentuates its cytolytic action. Binding of the C5b 6-7-8-9 complex on the membrane changes the membrane structure and membrane charges considerably. The C5b-6-7-8-9 complex gets inserted into the membrane to produce cylindrical lesions of circular cross-section (8-12 nm in diameter) in the membrane. Each such lesion either forms a transmembrane protein channel across the lipid bilayer of the membrane or changes the molecular organization of membrane lipids to produce a transmembrane lipid channel. Such transmembrane channels are permeable to small solutes such as electrolyte ions (e.g., Na^+) and water. So, the membrane lesion is followed by an influx of Na^+ , other electrolyte ions and water—the influx of water is due to the higher colloidal osmotic pressure inside the cell than outside it. The osmotic influx of water ultimately causes the lysis of the cell. Complements are of special importance in the lysis of Gram-negative bacterial cell whose cell wall is resistant to lysozyme action.

(b) *Anaphylactic and inflammatory effects*: Small complement fractions C3a, C4a and C5a, produced at intermediate steps of the complement pathway, act as *anaphylatoxins*. They act on mast cells and basophils to trigger the release of anaphylactic and inflammatory chemicals (e.g., histamine, neutrophil chemotactic factor, eosinophil chemotactic factor and platelet activating factor) from the granules of those cells; but these complements do not act through the receptors for IgE on the membranes of those cells. The substances released from those cells produce in turn inflammation and anaphylaxis to help in body immunity. Besides acting as anaphylatoxins, C3a, C4a and C5a can also promote inflammation by direct effects in enhancing vascular permeability, release of lysosomal enzymes from granulocytes, and slow sustained contractions of smooth muscles like those of intestines and bronchi.

(c) *Chemotactic action*: C5a as well as C5b-6-7 complex causes chemotaxis of neutrophil leucocytes towards the antigenic cell. Thus, it causes infiltration and aggregation of neutrophils in the affected tissue.

(d) *Respiratory burst in leucocytes*: C5a stimulates oxidative metabolism in leucocytes and thereby produces the respiratory burst, i.e., the sudden manifold rise in O_2 -consumption seen in those cells during phagocytosis. This helps in the formation of oxygen metabolites like superoxide anions (O_2^-), singlet oxygen ($^1\text{O}_2$) and free hydroxyl radical ($\cdot\text{OH}$) which help to kill and destroy the phagocytosed microbe.

(e) *Immune adherence*: C3b produced during complement activation promotes the phagocytosis of antigenic cells by phagocytes by causing the adherence of the antigenic cell on the phagocyte surface. This process, called immune adherence, consists of the linking of the two cells by C3b molecules. Many C3b molecules bind on one hand to the antigenic cell surface and on the other hand, to specific C3b receptors on the phagocyte plasma membrane.

9.11 LYMPHOKINES

These are heterogeneous soluble substances produced and released by some specifically sensitized T lymphocytes (helper T cells) following their initial reaction with antigens. These lymphokines help in cell-mediated immunity by mediating in the reactions involving diverse types of cells such as macrophages, lymphocytes, granulocytes and antigen-bearing cells. Some of the better known lymphokines are mentioned below.

(a) *Lymphokines affecting macrophages*: These factors affect the macrophages and recruit them for action against antigenic materials. For example, (i) *macrophage chemotactic factor*, a protein of MW 12-25 kdal, selectively attracts mononuclear phagocytes (macrophages and monocytes) and causes

their chemotactic migration to the site of T cell activation; (ii) *macrophage inhibitory factor* (MIF), a glycoprotein of MW 23-55 kdal, binds with fucose-containing membrane glycolipids on macrophages to change their membrane properties, thereby making them adhere together and preventing their dispersion from the site of inflammation; (iii) *macrophage activating factor* (MAF) stimulates the macrophages to enhance their cellular adherence, membrane adenylate cyclase activity, number and motility of cytoplasmic processes, ruffled membrane activities, membrane glycoprotein synthesis, pentose phosphate pathway, and phagocytic, bacteriostatic and tumoricidal activities; (iv) *skin reactive factor* evokes an erythematous reaction in the skin, enhances capillary permeability and extravasation of fluid, and facilitates the recruitment of circulating monocytes as extravascular macrophages.

(b) *Growth and differentiation factors*: These include various lymphokines affecting growth and differentiation of diverse types of cells such as lymphocytes, macrophages, neutrophil leucocytes, mast cells and megakaryocytes. For example, (i) *interleukin-2* (IL-2), a monomeric peptide of MW 15.5 kdal, binds with IL-2 receptors on T lymphocytes to cause their proliferation into a specific T cell population; (ii) *interleukin-3* (IL-3) acts as a hemopoietin and stimulates the proliferation and recruitment of erythroid cells, macrophages, mast cells and megakaryocytes; (iii) *GM-CSF* stimulates the proliferation and differentiation of macrophages and neutrophil leucocytes from undifferentiated stem cells in the bone marrow; (iv) *B lymphocyte stimulating factor* (BSF-I) causes activation, growth and proliferation of B lymphocytes into clones of B cells; (v) *B cell growth factor II* (BCGF-II) acts after the action of BSF-I, stimulates the activated B lymphocytes to proliferate and thereby further expands the clone of B cells; (vi) *B cell*

differentiation factors (BCDF) promote the differentiation of activated B lymphocytes of the expanded clones to produce plasma cells secreting a specific class of immunoglobulins and also stimulate the *class switch* of plasma cells secreting one class of immunoglobulins (say, IgM) to plasma cells secreting a different class of immunoglobulins (say, IgG).

(c) *Cytotoxic lymphokine*: Some T lymphocytes secrete *lymphotoxin* which inactivates and kills many types of cells including fibroblasts, tumor cells, other transformed cells and even T lymphocytes. $\text{IFN}\gamma$ or γ interferon helps in this cytotoxic action of lymphotoxin by enhancing the density of lymphotoxin receptors on the membrane of those cells.

(d) *Interferons (IFN)*: These are low-MW proteins or glycoproteins, at least some of which (e.g., $\text{IFN}\gamma$ and $\text{IFN}\alpha$) are released by sensitized lymphocytes. They activate and induce the killer cells to enhance their cytotoxic and cytolytic actions on virus-infected host cells, inhibit the synthesis of viral proteins in the host cell to block the replication of viruses, prevent a second viral attack from being superimposed on an animal already infected with a virus, and exert a tumor-regressor effect by inhibiting growth and proliferation of tumor cells.

9.12 BLOOD GROUPS

The erythrocyte membrane carries different antigens called *blood group antigens* or *agglutinogens*. The agglutinogen on the erythrocyte membrane of one individual may react with a specific serum antibody occurring in the blood of some other individual of the same species. This results in the clumping or agglutination of the erythrocytes and their hemolysis. Such antibodies are called *agglutinins* or *hemagglutinins*. They are either inherited or produced on exposure to antigens of erythrocytes of some other person.

The human race is divided into *blood groups* according to the agglutinogens on the erythrocytes. More than 20 blood group systems have been described, each consisting of several structurally related agglutinogens.

ABO blood group system

This consists of 4 major blood groups, viz., A, B, AB and O groups, on the basis of the presence or absence of two antigens called A and B antigens on the erythrocyte membrane. These two antigens also occur on the membranes of epithelial and endothelial cells of salivary glands, pancreas, liver, lungs, kidneys and testes.

Plasma membranes of erythrocytes and other cells carry A antigen in group A persons, B antigen in group B, both A and B antigens in group AB, and neither of the two in group O. In addition, H antigen occurs in the erythrocyte membranes of all groups except the Bombay O subgroup of group O. The A group is subdivided mainly into A_1 and A_2 subgroups. About $\frac{4}{5}$ ths of A group persons belong to the A_1 subgroup and have both A and A_1 antigens on the erythrocyte membrane; but about $\frac{1}{5}$ th of A group individuals possess only the A antigen and little or no A_1 antigen, and they are included in the A_2 subgroup. The AB group is also divided mainly into A_1B and A_2B subgroups— A_1B subgroup individuals have A, A_1 and B antigens on their erythrocyte membrane, but A_2B subgroup persons have only A and B antigens. A_1B subgroup includes majority of AB group individuals. H antigen is high in amount in the O group, absent in the Bombay O subgroup, insignificantly low in A_1 , A_1B and B groups, and low but significant in amount in A_2 group. Most of the individuals of all groups are *secretors* and secrete their blood group antigens in their saliva, gastrointestinal secretions, semen, milk, urine and respiratory tract secretions. But a small number of *nonsecretors* do not secrete their blood group antigens in these secretions.

TABLE 9.9. The ABO blood group system.

Blood group	Agglutinogens on erythrocyte	Agglutinins in plasma
A_1	A, A_1	Anti-B, anti-H
A_2	A, (H)	Anti-B, (anti- A_1)
B	B	Anti-A, anti-H
A_1B	A, A_1 , B	Anti-H
A_2B	A, B	(Anti- A_1)
O	H	Anti-A, anti-B
Bombay O	—	Anti-A, anti-B, anti-H

Anti-A (α) and anti-B (β) agglutinins are inherited and occur naturally in the serum of groups B and A respectively. Anti-A agglutinin reacts with the A antigen of A_1 , A_2 , A_1B and A_2B group erythrocytes and consequently agglutinates those cells. Anti-B agglutinin similarly agglutinates the erythrocytes of B, A_1B and A_2B groups by reacting with the B antigen of those cells. Anti- A_1 agglutinin has been demonstrated in the sera of some individuals of A_2 and A_2B groups; it does not react with A antigen but agglutinates A_1 and A_1B cells by reacting with their A_1 antigen. Group O serum contains both anti-A and anti-B agglutinins while the sera of A_1B and A_2B groups contain neither of these two (Table 9.9). Anti-H agglutinin occurs in the sera of A_1 , A_1B , B and Bombay O groups and agglutinates O and A_2 group erythrocytes by reacting with their H antigen. A person normally carries such agglutinins in the serum as do not react with the antigens of his own erythrocytes. These agglutinins are mainly IgM immunoglobulins, to a lesser extent IgG immunoglobulins and much less frequently IgA immunoglobulins. IgM agglutinins are more agglutinating than hemolytic in effect; IgG agglutinins can cross the placental membranes and are more hemolytic than IgM. Fetal and neonatal sera have low titres of agglutinins, mostly maternal IgG agglutinins transferred across the placenta. Synthesis of agglutinins starts after birth

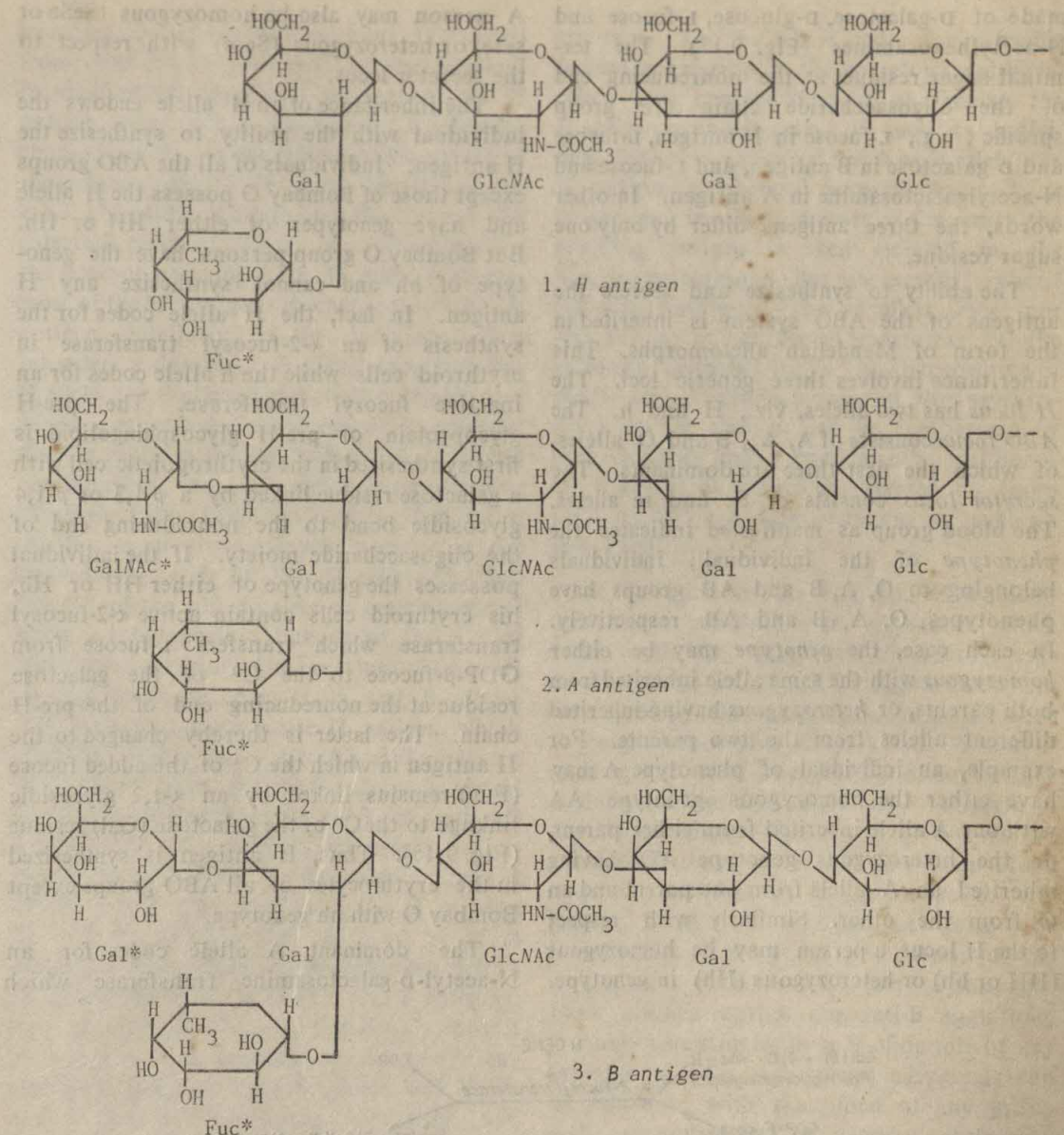


Fig. 9.17. Oligosaccharide chains of H, A and B antigens. * indicates group-specific sugars.

probably due to immunization against extrinsic antigens having antigenic determinants similar to such blood group antigens as are normally absent in that individual.

A, B and H antigens of cell membranes are either glycoproteins or glycosphingolipids,

but those secreted are mostly glycoproteins; for example, the antigens secreted in gastrointestinal and bronchial secretions consist of oligosaccharides bound to the mucins (mucoproteins) of those fluids. The oligosaccharide parts of A, B and H antigens are

made of D-galactose, D-glucose, L-fucose and N-acetylhexosamines (Fig. 9.17). The terminal sugar residues at the nonreducing end of the oligosaccharide chain are group specific; e.g., L-fucose in H antigen, L-fucose and D-galactose in B antigen, and L-fucose and N-acetylgalactosamine in A antigen. In other words, the three antigens differ by only one sugar residue.

The ability to synthesize and secrete the antigens of the ABO system is inherited in the form of Mendelian allelomorphs. This inheritance involves three genetic loci. The *H locus* has two alleles, viz., H and h. The *ABO locus* consists of A, A₁, B and O alleles, of which the first three are dominants. The *secretor locus* consists of Se and se alleles. The blood group as manifested indicates the *phenotype* of the individual; individuals belonging to O, A, B and AB groups have phenotypes O, A, B and AB respectively. In each case, the *genotype* may be either *homozygous* with the same allele inherited from both parents, or *heterozygous* having inherited different alleles from the two parents. For example, an individual of phenotype A may have either the homozygous genotype AA with one A allele inherited from either parent, or the heterozygous genotype AO having inherited an A allele from one parent and an O from the other. Similarly with respect to the H locus, a person may be homozygous (HH or hh) or heterozygous (Hh) in genotype.

A person may also be homozygous (SeSe or sese) or heterozygous (Sese) with respect to the secretor locus.

The inheritance of an H allele endows the individual with the ability to synthesize the H antigen. Individuals of all the ABO groups except those of Bombay O possess the H allele and have genotypes of either HH or Hh. But Bombay O group persons have the genotype of hh and cannot synthesize any H antigen. In fact, the H allele codes for the synthesis of an α -2-fucosyl transferase in erythroid cells while the h allele codes for an inactive fucosyl transferase. The pre-H glycoprotein or pre-H glycosphingolipid is first synthesized in the erythropoietic cell with a galactose residue linked by a β -1,3 or β -1,4 glycosidic bond to the nonreducing end of the oligosaccharide moiety. If the individual possesses the genotype of either HH or Hh, his erythroid cells contain active α -2-fucosyl transferase which transfers L-fucose from GDP- β -fucose to the C² of the galactose residue at the nonreducing end of the pre-H chain. The latter is thereby changed to the H antigen in which the C¹ of the added fucose (Fuc) remains linked by an α -1,2 glycosidic linkage to the C² of the galactose (Gal) residue (Fig. 9.18). Thus, H antigen is synthesized in the erythrocytes of all ABO groups except Bombay O with hh genotype.

The dominant A allele codes for an N-acetyl-D-galactosamine transferase which

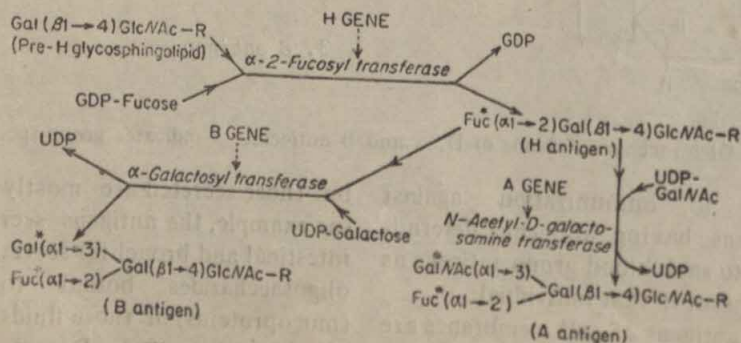


Fig. 9.18. Synthesis of H, A and B antigens.

transfers N-acetyl-D-galactosamine (GalNAc) from UDP-GalNAc to the C³ of the terminal fucose-carrying galactose residue of the H antigen (Fig. 9.18). The latter is thereby changed to the A antigen in which the C¹ of the added GalNAc remains bound by an α -1,3 glycosidic linkage to the C³ of the galactose residue. Thus, in erythrocytes of the A group carrying both H and A alleles, most of the H antigen is changed to the A antigen. Similarly, if the individual has inherited a dominant B allele along with the H allele, the B allele codes for the translation of an α -galactosyl transferase which changes the H antigen to the B antigen; the enzyme accomplishes this change by transferring galactose from UDP-galactose to the C³ of the fucose-carrying galactose residue at the nonreducing end of the H antigen so that the C¹ of the added galactose remains bound by an α -1,3 glycosidic linkage to the C³ of the fucose-carrying Gal residue. The AB group individuals carry H, A and B alleles and consequently synthesize H antigen which is almost totally changed to A and B antigens. This also explains why the H antigen is absent or poor in the erythrocytes of A, B and AB groups. On the contrary, O group individuals inherit the H allele but neither A nor B allele; so they can synthesize the H antigen but cannot change the latter to A or B antigen. Thus, their erythrocytes are rich in the H antigen, but lack both A and B antigens. However, Bombay O group individuals inherit hh genotype and consequently fail to synthesize the H antigen; they, therefore, lack H, A and B antigens even though they may have inherited A and B alleles. The conversion of H antigen to A antigen is more exhaustive in A₁ and A₁B subgroups than in A₂ subgroup; so, H antigen is higher in the latter than in A₁ or A₁B subgroups.

Secretors belonging to any of the groups carry the Se allele either as the homozygous SeSe genotype or as the heterozygous Sese

genotype. The Se allele codes for a specific fucosyl transferase in the secretory epithelial cells. This enables them to synthesize the H antigen in those cells. If such an individual has also inherited the A or B allele, the H antigen is changed respectively to A or B antigen in the secretory cells and the resulting antigen is then secreted in the exocrine secretions. But nonsecretors inherit the sese genotype. The sese allele does not code for any active fucosyl transferase in the secretory epithelial cells. So, irrespective of the H, A or B antigen present in the erythrocytes, no H antigen can be synthesized in their secretory cells; consequently, neither A nor B antigen is formed or secreted by those cells in the exocrine secretions of such an individual.

If the serum of the recipient of a blood transfusion contains an agglutinin which reacts with the antigen on the donor's erythrocytes, the latter will be agglutinated and hemolyzed by the recipient's serum. Because O group erythrocytes carry neither A nor B antigen, the cells of a group O donor are not agglutinated by the serum of a recipient of any blood group. So, group O donors are considered *universal donors* and their blood may be transfused to the recipients of any group. Besides, a recipient can also be transfused with the blood of a donor of his own group. Because AB group serum contains neither anti-A nor anti-B agglutinin, it cannot agglutinate the cells of donors of any other group. So, AB group individuals can be transfused with the blood of any group and are considered as *universal recipients*. But such indiscriminate transfusions are not advisable because the bloods of the donor and the recipient may still be incompatible due to the presence of antigens other than those of the ABO system. Group O plasma should not be transfused in large amounts to A or B group recipients because O group plasma carries stronger anti-A and anti B

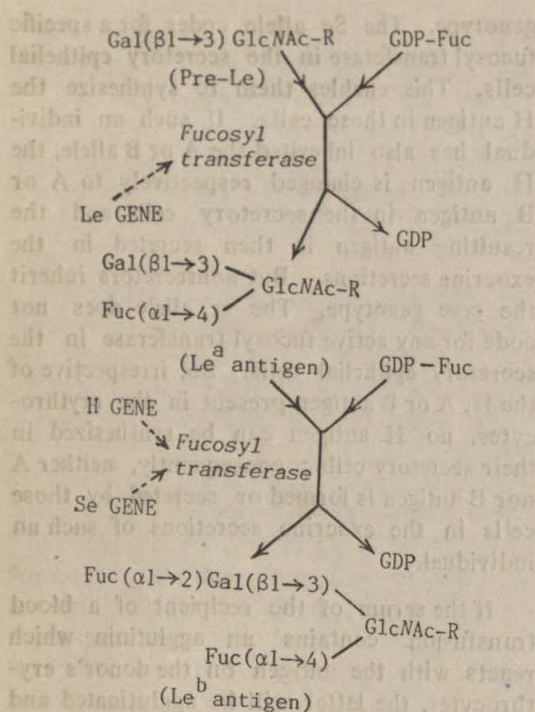


Fig. 9.19. Synthesis of Lewis antigens.

agglutinins which may agglutinate the recipient's cells.

Lewis blood group system

Recognized Lewis antigens comprise Le^a, Le^b, Le^c, Le^d and Le^x. They are glycosphingolipids. They may circulate in the plasma in association with HDL and LDL lipoproteins, get adsorbed on erythrocytes and in secretors, are secreted in the saliva. Their oligosaccharide moieties closely resemble those of ABO group antigens and are made of Fuc, Gal, Glc, GlcNAc and GalNAc. Their stem carbohydrate or pre-Le chain is identical in structure with that of the pre-H chain. If the individual has inherited an Le gene, the latter codes for a fucosyl transferase which transfers a fucose from GDP-β-fucose to the penultimate GlcNAc residue next to the terminal galactose residue at the nonreducing end of the pre-Le chain, changing the latter

to Le^a (Fig. 9.19). If the individual is a nonsecretor and lacks the Se allele, this Le^a is his major Lewis antigen—about 20% of humans are Le^a(+). If the individual is a secretor and has inherited the Se allele (probably along with the H allele), he synthesizes another fucosyl transferase which adds a second fucose from GDP-β-fucose to the terminal galactose residue, changing the molecule to Le^b—about 75% of humans are Le^b(+). The remaining 5% of the population are Le^{a-b-}. The antigenic specificity of Le^a depends on the terminal galactose and fucose residues of its oligosaccharide chain; the specificity depends on the two terminal L-fucose residues in case of Le^b. Weak anti-Lewis antibodies, mostly IgM immunoglobulins, may circulate naturally only in Le^{a-b-} individuals. Anti-Le^b antibodies create no transfusion problem. But sometimes strong anti-Le^a antibodies may cause adverse transfusion reactions.

Rh system

This system consists of 26 antigens such as D, C, E, e, d and c. Of these, D is the strongest and the only major Rh antigen. Rh antigens occur in erythrocyte membrane, but neither in the plasma, nor in secretions like saliva. Persons with D antigen on the erythrocyte are placed in the Rh(+) group; those lacking that antigen are included in the Rh(-) group. More than 96% of Indians have Rh(D) antigen and belong to the Rh(+) group. But 12-17% of Chitrapur Saraswat Brahmins of Udipi and Parsis are Rh(-).

Except anti-E agglutinins in some cases, no anti-Rh agglutinin normally occurs in the plasma. But anti-D agglutinins may be produced in an Rh(-) mother either due to a transfusion with D-positive blood or due to a leakage of D antigen of an Rh(+) fetus across placental barriers. Anti-D agglutinins are mainly IgG₁ and IgG₃ and only occasionally IgM. The anti-D IgG may again pass

from the maternal to the fetal blood, usually in subsequent pregnancies following the immunization, and agglutinate the erythrocytes of the Rh(+) fetus, causing hemolysis, anemia, jaundice, edema, and even death of the fetus (*hemolytic disease of the new-born*). Sometimes, such anti-D mediated hemolysis in the fetus leads to the deposition of bile pigments in the corpus striatum and other basal ganglia with neurological symptoms (*kernicterus*). Passive administration of *anti-D* (*Rh₀*) *immunoglobulins* to the Rh(-) mother after the birth of the first Rh(+) baby may inhibit Rh-immunization of the mother and prevent the rise in the serum anti-D antibody titre in her blood; this reduces the incidence of the hemolytic disease in her subsequent children.

9.13 PORPHYRINS AND HEMOGLOBIN

Porphyrins are colored organic compounds, each consisting of a tetrapyrrole super-ring composed of 4 pyrrole rings linked by methenyl bridges. The tetrapyrrole super-ring may carry a metal ion such as Fe^{2+} , Fe^{3+} or Mg^{2+} , bound to the nitrogens of its pyrrole rings. Besides, different porphyrins carry different sidechains substituted for 8 hydrogens of the 4 pyrrole rings. These substituent sidechains include the following: methyl (M): $-\text{CH}_3$; vinyl (V): $-\text{CH}=\text{CH}_2$; acetate

(A): $-\text{CH}_2\text{COOH}$; propionate (P): $-\text{CH}_2-\text{CH}_2\text{COOH}$. In *type I porphyrins*, these sidechains are arranged symmetrically on all four pyrrole rings; e.g., coproporphyrin I and uroporphyrin I (Fig. 9.20); but in *type III porphyrins* such as coproporphyrin III, uroporphyrin III and protoporphyrin III, the substituents are arranged asymmetrically because the expected order of the substituents is reversed in the pyrrole ring IV.

Porphyrins, dissolved in organic solvents or in HCl, give a characteristic *red fluorescence* in ultraviolet light due to their double bonds. Porphyrins in HCl solution show characteristic *absorption bands* in both UV and visible wavelengths of the spectrum. One of the bands, called the *Soret band*, is located near 400 nm and is exhibited by all porphyrins. Other absorption bands are characteristic of the respective porphyrins according to their substituent sidechains. Absorption bands of a porphyrin in the visible wavelengths may undergo changes on the binding of the porphyrin with a metal ion.

Metalloporphyrins such as the iron-porphyrins like heme can bind to specific proteins. For example, the Fe^{2+} ion held by the pyrrole nitrogens of heme may be coordinately linked to the imidazole-N of specific histidine residues of particular apoproteins to form

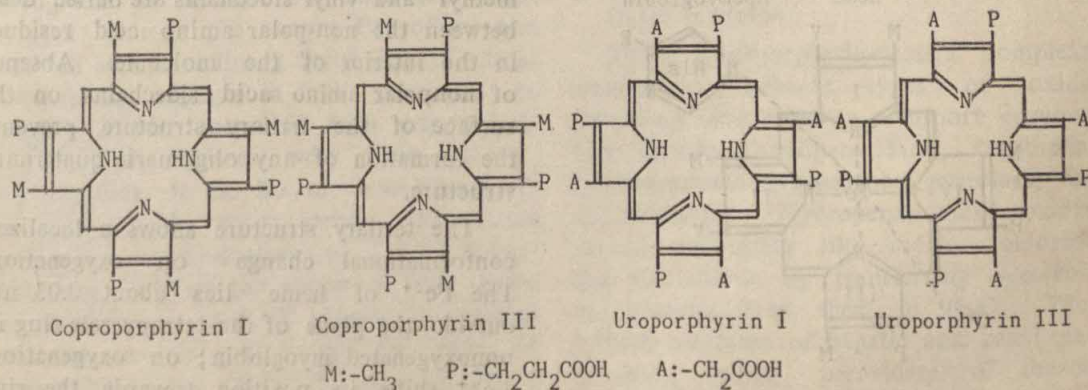


Fig. 9.20. Types I and III of porphyrins.

different *hemoproteins* such as hemoglobin, cytochromes, myoglobin and heme enzymes.

Myoglobin

Myoglobin is a hemoprotein present in rich amounts in red skeletal muscle fibres. Adult human beings have about 40 g of myoglobin accounting for about 10% of the total body heme. Avian muscles for sustained flight and muscles of diving aquatic mammals have higher myoglobin contents than human muscles.

Structure :

Myoglobin (MW 17 kdal) consists of a single peptide chain (apomyoglobin) of 153 amino acid residues and a single heme molecule whose Fe^{2+} is coordinately linked with the imidazole-N of a specific histidine residue of apomyoglobin (Fig. 9.21). The heme of myoglobin is identical with that of hemoglobin. Myoglobin has a spheroidal globular molecule of dimension about $4.5 \times 3.5 \times 2.5$ nm. The nonpolar amino acid residues such as Val, Met, Leu and Phe are located in the interior of the compact globular molecule, polar amino acids are exposed on the surface of the molecule, and amino acids with both polar and nonpolar parts

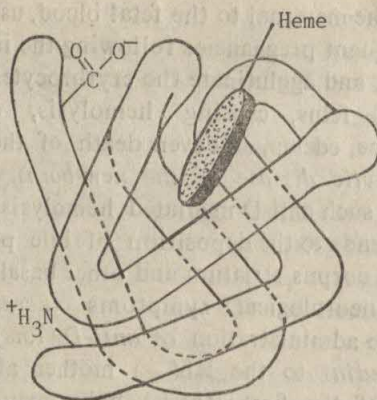


Fig. 9.22. Tertiary structure of myoglobin.

such as Tyr and Trp have their nonpolar parts hidden in the interior of the molecule.

Myoglobin has a *secondary structure* consisting of 8 α -helical segments, each of 7-20 amino acid residues, and short non-helical random segments alternating with the α -helices. Some of the nonhelical segments accommodate proline residues.

The molecule is folded at its non-helical regions to wind about itself, producing a *tertiary structure* with the heme buried in a hydrophobic pocket between two α -helices (Fig. 9.22). The polar propionate sidechains of pyrroles III and IV of heme are located on the surface of the myoglobin molecule while the pyrrole rings and their nonpolar methyl and vinyl sidechains are buried deep between the non-polar amino acid residues in the interior of the molecule. Absence of nonpolar amino acid sidechains on the surface of the tertiary structure prevents the formation of any oligomeric quaternary structure.

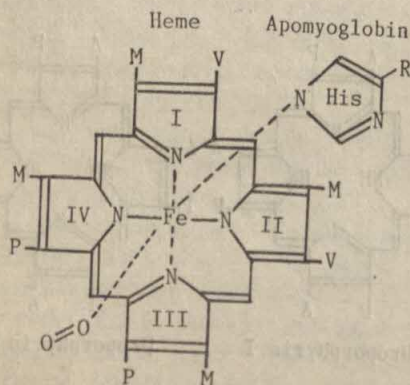


Fig. 9.21. Prosthetic group of oxy-myoglobin linked to apomyoglobin.

The tertiary structure shows a localized conformational change on oxygenation. The Fe^{2+} of heme lies about 0.03 nm outside the plane of the tetrapyrrole ring in nonoxygenated myoglobin; on oxygenation, Fe^{2+} shifts its position towards the ring along with the histidine residue connected

with it, pulling the adjoining part of the peptide chain also towards the ring. The oxygen remains coordinately bound to Fe^{2+} in oxy-myoglobin.

Kinetics of oxygenation :

Myoglobin stores much oxygen as oxy-myoglobin in the red muscle fibres and releases the oxygen at very low oxygen tensions (Po_2) like 5 mm Hg as happens in red fibres during sustained work. This enables these fibres to carry out aerobic contractions for prolonged periods without the accumulation of lactic acid. But myoglobin is not suitable for oxygen transport because (i) it has a much higher oxygen-affinity than hemoglobin and cannot deliver its oxygen even at the low Po_2 of 20 mm Hg in ordinarily active tissues, and (ii) its oxygen-affinity is not lowered by a rise in Pco_2 in active tissues.

These properties of myoglobin are demonstrated in its oxygen saturation (dissociation) curve (Fig. 9.23). This curve is obtained by plotting the percent O_2 -saturation of myoglobin against Po_2 . The oxygen dissociation curve of myoglobin is hyperbolic in shape in contrast to the sigmoid shape of the oxygen dissociation curve of hemoglobin. The hyperbolic dissociation curve of myoglobin shows that at the arterial Po_2 of about 95 mm Hg, myoglobin is about 90% saturated with oxygen. But at the venous Po_2 of 40 mm Hg and at the ordinary tissue Po_2 of about 20 mm Hg, myoglobin still continues to remain 80-85% saturated with oxygen and consequently releases very little oxygen; nevertheless, at the Po_2 of about 5 mm Hg or lower in the active muscle, its oxygen saturation sharply declines to 20% or lower, releasing much of the oxygen so long stored in combination with it. Unlike the oxygen dissociation curve of hemoglobin, that of myoglobin is not shifted to the right by a rise in Pco_2 —this indicates that a rise in Pco_2

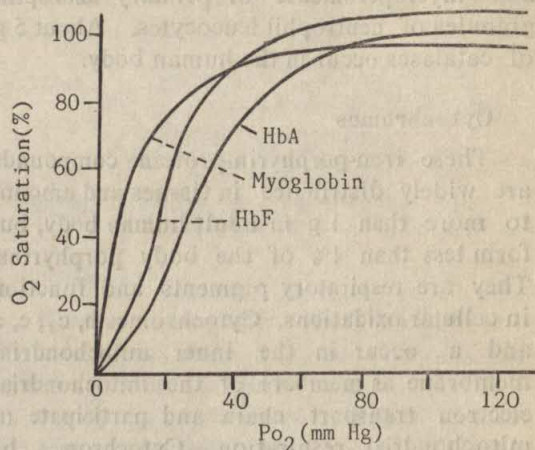


Fig. 9.23. Oxygen dissociation curves of myoglobin, hemoglobin A and hemoglobin F.

cannot lower the oxygen-affinity of myoglobin; in other words, there is *no Bohr effect* of Pco_2 on oxy-myoglobin.

The hyperbolic form of the oxygen dissociation curve of myoglobin indicates the *absence of cooperativity* between oxygen molecules in binding with myoglobin—binding of one O_2 molecule with myoglobin does not change or enhance the ability of the latter to bind with further O_2 molecules. This lack of cooperativity results from the monomeric form of myoglobin with a single Fe^{2+} .

Heme enzymes

These iron-porphyrin-protein complexes function as different types of oxidoreductases and electron transport enzymes. They include hydroperoxidases, tryptophan 2,3-dioxygenase (tryptophan pyrrolase) and cytochromes. Hydroperoxidases oxidize various substrates like iodide, chloride, and glutathione by transferring electrons and protons from them to H_2O_2 . They include catalases of hepatic and renal peroxisomes, iodine peroxidase of thyroid cells, glutathione peroxidase of erythrocytes,

Cytochromes a and *a₃* remain associated with each other to constitute an enzyme called *cytochrome oxidase* (MW 200 kdal). Both the cytochromes remain bound to the same apoprotein to form the enzyme. The enzyme contains two heme A molecules (Fig. 9.25), each with a farnesyl sidechain on pyrrole I and a formyl sidechain on pyrrole IV. It also contains two copper atoms. Ferrocycytochrome *aa₃*, unlike other ferrocycytochromes, can transfer electrons directly to molecular *O₂*, but is inhibited by cyanides and CO.

Cytochrome b₂ is a hemoflavoprotein—its molecule is made of flavin mononucleotide (FMN), iron-protoporphyrin III (heme) and protein. The electrons accepted by cytochrome *b₂* flow between the metal ion and the flavin before being finally donated to the next electron-acceptor.

Cytochromes b₆ and *P₄₅₀* act as electron carriers in the smooth endoplasmic reticulum membrane to participate in hydroxylations.

Hemoglobins

These are oxygen-carrying pigments of blood. They occur inside the erythrocytes in mammals, birds, reptiles, amphibians and fishes, but in the plasma in many molluscs and annelids. The segregation of hemoglobin within erythrocytes enables the vertebrates to carry large amounts of it in the blood without any rise in plasma osmotic pressure or viscosity. Each decilitre (100 ml) of human blood contains about 15 g of hemoglobin (Hb). About 88% of the total body heme occurs in hemoglobin. *Mean corpuscular Hb* (MCH) is the average amount of Hb per erythrocyte; it amounts to about 29 picograms (pg) in normal man, upto about 50 pg in macrocytic anemia, and as low as 15 pg in hypochromic anemia. *Mean corpuscular Hb concentration* (MCHC) is the concentration of Hb per unit volume of erythrocytes; it amounts to about 35% in normal man and as low as 20% in

hypochromic anemia, but may remain unaltered in macrocytic anemia.

$$\begin{aligned} \text{MCH} &= \frac{\text{Amount of Hb per litre of blood}}{\text{Number of erythrocytes per litre of blood}} \\ &= \frac{10 \times \text{Hb concentration}}{\text{Erythrocyte count in millions}} \\ \text{MCHC} &= \frac{100 \times \text{Hb concentration per dl}}{\text{Hematocrit value}} \end{aligned}$$

Structure :

Human hemoglobin (MW 64450) is a chromoprotein made of an apoprotein called *globin* and a prosthetic group of *Fe²⁺-protoporphyrin III* compound called *heme*. Globin is a tetrameric basic protein of histone class. It is made of paired peptides of two different types; α , β , γ , δ , ϵ and other types of peptides have been identified in human hemoglobins. Each of the four peptide chains remains bound to a heme molecule which is identical in all hemoglobins. Heme bears methyl and vinyl sidechains on pyrroles I and II, and methyl and propionate sidechains on pyrroles III and IV.

Normal human hemoglobin has its globin part made of pairs of α and β peptide chains. The α and β chains differ in their *primary structures* which are constituted by the linear chains of 141 and 146 amino acid residues respectively; they possess MWs of 15126 and 15866 daltons respectively. The *secondary structure* of each chain consists of 7 α -helical segments alternating with short, nonhelical, randomly coiled segments. The *tertiary structure* of each peptide results from the folding of its chain at the nonhelical segments; nonpolar amino acid residues are located deep inside the coils while polar amino acid residues are mostly on the surface of the coils. The tertiary structure buries in one of its hydrophobic pockets a heme molecule surrounded by nonpolar amino acid sidechains. The *Fe²⁺* ion of the heme molecule is coordinately linked to the imidazole-N of two histidine residues of the peptide chain

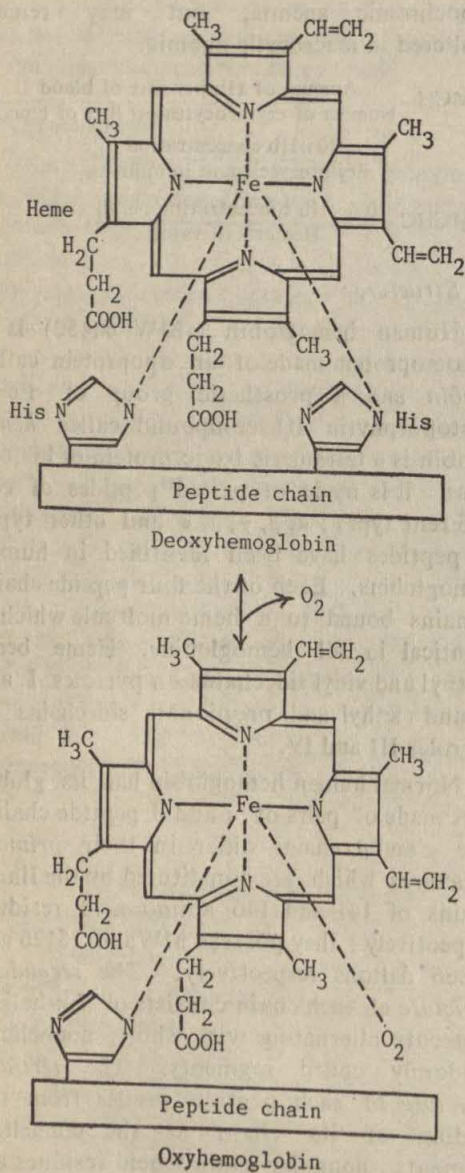


Fig. 9.26. Oxyhemoglobin and deoxyhemoglobin.

(Fig. 9.26). The quaternary structure of hemoglobin consists of a globular tetrameric molecule ($5 \times 5.5 \times 6.4$ nm) formed by the noncovalent binding of two pairs of peptide chains, each with its individual folded and coiled secondary-tertiary structure and its own heme prosthetic group (Fig. 9.27). The

peptide chains are held together and the quaternary structure is stabilized by many hydrophobic bonds, some hydrogen bonds and also some electrostatic bonds (salt bridges). Because the molecule is made of four peptide chains, each holding a heme molecule, hemoglobin contains 4 gram atoms of Fe^{2+} per mole.

Different types of hemoglobin differ in their quaternary structure according to the monomer units or peptide chains constituting their molecules. Normally, about 98% of the total hemoglobin of adult humans consists of hemoglobin A (HbA) with its globin made of two α and two β peptide chains and having the quaternary structure of $\alpha_2\beta_2$. Electrostatic bonds or salt bridges, involving the C-terminals of all four peptide chains, hold the quaternary structure of deoxygenated or partially oxygenated HbA in a "taut" or *T* form; with the progressive oxygenation of the four subunits of HbA, these salt links are progressively weakened and broken, causing a transition of the quaternary structure from the *T* form to a "relaxed" or *R* form (Fig. 9.28). About 4% and 1% of HbA remain glycosylated as Hb1b and Hb1c respectively. Both these possess the quaternary structure of $\alpha_2\beta_2$, but the N-terminal amino acids of

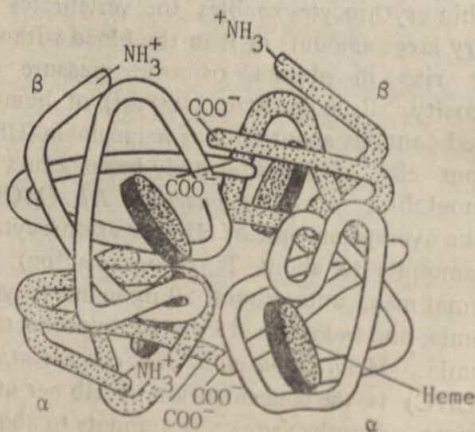


Fig. 9.27. Tertiary-quaternary structure of hemoglobin.

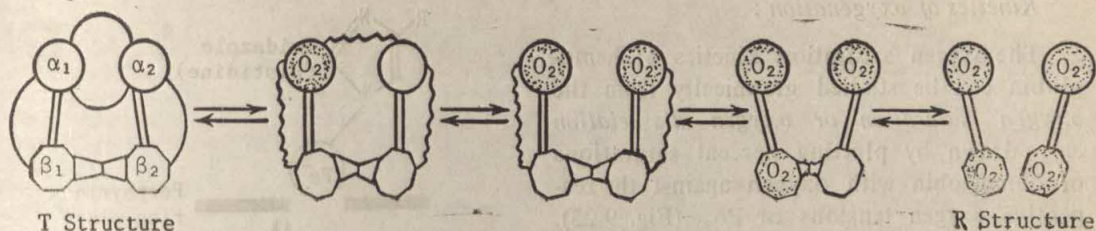


Fig. 9.28. Change of T form of hemoglobin to R form on progressive oxygenation.

the β chains remain bound to specific sugars; Hb ϵ bears, for example, a glucose residue bound to the terminal Val residue of each β chain.

Only about 2.5% of adult hemoglobin consists of *hemoglobin A₂* (HbA₂) having $\alpha_2\delta_2$ as its quaternary structure; each of the two δ chains which replace the β chains in HbA₂, possesses the same total number of 146 amino acid residues as the β chain, but differs from the latter with respect to the nature of ten of those amino acids.

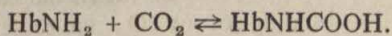
Embryonic hemoglobins occur in human erythrocytes in the first 3 months of embryonic life. They include *Gower I* and *Gower II* hemoglobins with the quaternary structures of $\zeta_2\epsilon_2$ and $\zeta_2\gamma_2$ respectively. Later in fetal life, embryonic hemoglobins are replaced by *fetal hemoglobin* (HbF) containing a globin with the quaternary structure of $\alpha_2\gamma_2$. Each of the two γ chains which replace the β chains in HbF, consists of the same total number of 146 amino acid residues as the β chain, but differs from the latter with respect to the nature of 37 of those amino acids. HbA appears in the fetal blood by the 20th week of the fetal life and progressively increases to form 20% and 90% of the total circulating hemoglobin at birth and 4 months after birth respectively.

Reactions :

Hemoglobin can form a loose complex with molecular O_2 to become oxyhemoglobin

(HbO₂). During such oxygenation, each of its four Fe^{2+} ions opens one of its coordinate bonds with the imidazole-N of globin and binds with a molecule of O_2 instead; thus, fully saturated oxyhemoglobin carries 4 O_2 . At low P_{O_2} , oxyhemoglobin dissociates into molecular O_2 and reduced or deoxyhemoglobin (Hb).

Hemoglobin, particularly deoxyhemoglobin, combines with CO_2 at the NH_2 groups of the β chains in particular, to form *carbaminohemoglobin* :



CO has a higher affinity than O_2 for hemoglobin and may combine with the latter, displacing O_2 , to form a relatively stable, photolabile, cherry-red complex called *carboxyhemoglobin*.

Ferricyanide, peroxides, ozone, nitrates or permanganate may oxidize Fe^{2+} to Fe^{3+} in hemoglobin, changing the latter to the brown compound called *methemoglobin*. Unlike hemoglobin, the latter cannot dissociate easily to release O_2 , cannot combine with CO , but can combine with cyanides and sulfides to give *cyanomethemoglobin* and *sulfmethemoglobin* respectively.

Deoxyhemoglobin and methemoglobin show *absorption bands* at λ 559 and 634 nm respectively. Oxyhemoglobin has three absorption bands at 425, 542 and 578 nm; carboxyhemoglobin exhibits two at 542 and 570 nm.

Kinetics of oxygenation :

The oxygen saturation kinetics of hemoglobin can be studied graphically from the oxygen saturation or oxygen dissociation curve drawn by plotting percent saturations of hemoglobin with oxygen against the respective oxygen tensions or P_{O_2} (Fig. 9.23). The oxygen dissociation curve of hemoglobin is sigmoid in shape, in contrast to the hyperbolic form of the oxygen dissociation curve of myoglobin. This sigmoid form of the oxygen saturation kinetics of hemoglobin is comparable with the sigmoid substrate saturation kinetics of allosteric enzymes (vide page 103). The sigmoid kinetics results from a cooperativity between specific ligand molecules binding with the protein. For such cooperativity between more than one ligand molecule, the protein must possess more than one binding site on its molecule. Hemoglobin, for example, possesses four oxygen-binding sites on its four subunits and a *positive cooperativity* exists between the ligand oxygen molecules—binding of an O_2 molecule with one of the O_2 -binding sites of hemoglobin increases its oxygen-affinity and facilitates the binding of subsequent O_2 molecules with other O_2 -binding sites of the same hemoglobin molecule. In contrast, oxygen molecules do not show cooperativity in binding with the monomeric molecule of myoglobin. Moreover, individual monomeric subunits of hemoglobin resemble myoglobin in possessing hyperbolic oxygen saturation curves. These indicate that the positive cooperativity between the ligand O_2 molecules is made possible by the tetrameric molecular structure of hemoglobin.

The Fe^{2+} ion of each heme ring lies about 0.06 nm outside the plane of that ring in deoxy-hemoglobin. During oxygenation, each Fe^{2+} opens one of its two coordinate bonds linked to the imidazole-N of two histidine residues of the peptide chain, and binds with an O_2

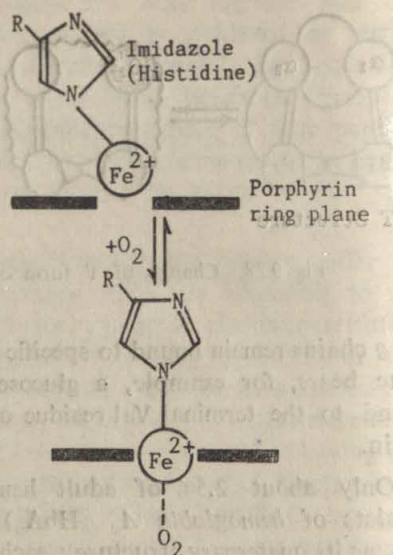


Fig. 9.29. Shift of Fe^{2+} of heme towards the plane of porphyrin ring on oxygenation.

molecule in place of that imidazole-N (Fig. 9.26). Binding with the O_2 molecule moves the Fe^{3+} towards the plane of its heme ring. The other histidine residue, still coordinately bound to the Fe^{2+} , is also pulled towards the heme ring (Fig. 9.29). The consequent pull on the peptide chain holding that heme unit strains and breaks some salt bridges between the peptide chains of the tetrameric Hb molecule. This enhances the likelihood of transition of the T form of its quaternary structure to the R form, more favourable for O_2 binding. It simultaneously changes the secondary-tertiary structures of the molecule, and rotates one $\alpha\beta$ pair of peptides relative to the other pair. These changes enhance the oxygen-affinity of the heme units and facilitate the binding of subsequent O_2 molecules to the Fe^{2+} ions of the hemes still remaining deoxygenated. At each step of oxygenation, these changes are further aggravated, progressively facilitating the binding of subsequent O_2 molecules with the remaining heme subunits until the hemoglobin molecule is fully saturated with 4 molecules of oxygen

and assumes the fully oxygenated R form (Fig. 9.28).

The sigmoid saturation kinetics enables hemoglobin to take up a maximal quantity of O_2 when exposed to high alveolar P_{O_2} in the lungs—hemoglobin becomes almost 97.5% saturated with O_2 at the alveolar P_{O_2} of 100 mm Hg. On the contrary, the same sigmoid kinetics enables oxyhemoglobin to release the maximal quantity of O_2 at low P_{O_2} in peripheral tissues—its O_2 saturation falls to about 75% in the mixed venous blood having a P_{O_2} of 40 mm Hg, and declines to about 20% in the capillaries of active muscles where the P_{O_2} has dropped to nearly 20 mm Hg.

A convenient index of the oxygen-affinity of hemoglobin is its P_{50} , which is the P_{O_2} at which hemoglobin is 50% saturated with O_2 . HbA normally has a P_{50} of 26 mm Hg. Oxygen-affinity is inversely proportional to P_{50} —the higher the P_{50} , the lower is the oxygen-affinity.

Rise in P_{CO_2} shifts the O_2 dissociation curve of hemoglobin to the right (Fig. 9.30), reduces the oxygen-affinity of hemoglobin, increases the P_{50} of hemoglobin and lowers its oxygen saturation, promoting the release of O_2 from oxyhemoglobin. This is called the *Bohr effect*. It is responsible for enhancing the release of oxygen from oxyhemoglobin in active tissues with high P_{CO_2} . The molecular basis of the Bohr effect is as follows. CO_2 is hydrated into H_2CO_3 by carbonic anhydrase inside erythrocytes. H_2CO_3 dissociates into HCO_3^- and H^+ . The protons (H^+) released from H_2CO_3 bind with the nitrogens of C-terminal histidine residues of the β chains of oxyhemoglobin. This protonation of C-terminal histidines of β chains helps to re-establish the salt bridges between the peptide chains of the hemoglobin molecule, favouring the transition of its R form with higher O_2 -binding capacity to the T form with much

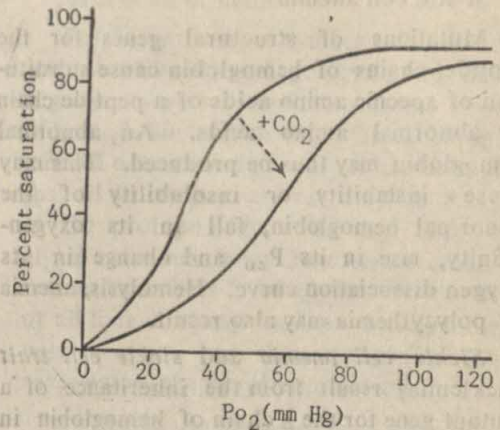


Fig. 9.30. Bohr effect of P_{CO_2} on the oxygen dissociation curve of hemoglobin A.

lower O_2 -binding capacity. This consequently promotes the release of O_2 from oxyhemoglobin.

Ascent to high altitudes, exercise, anemia, chronic hypoxia and alkalosis increase the concentration of 2,3-diphosphoglycerate (2,3-DPG) in erythrocytes. It is a highly charged anion and can bind with only the deoxygenated or T form of hemoglobin, but not with its oxygenated or R form. On binding with hemoglobin, it stabilizes the T form by cross-linking its β chains with additional salt bridges which must be disrupted for its transition to the R form. So, it reduces the oxygen-affinity of hemoglobin, raises its P_{50} , shifts the oxygen dissociation curve to the right and promotes the release of O_2 .

Fetal hemoglobin (HbF) has a higher oxygen-affinity and a lower P_{50} than HbA; the P_{50} of HbF is about 20 mm Hg. Its oxygen dissociation curve is steeper and to the left of that of HbA (Fig. 9.23). The higher O_2 -affinity of HbF results from a much weaker binding of 2,3-DPG to HbF and is helpful in oxygenation of fetal blood at the low P_{O_2} of maternal blood in the placenta.

Sickle cell anemia

Mutations of structural genes for the peptide chains of hemoglobin cause substitution of specific amino acids of a peptide chain by abnormal amino acids. An abnormal hemoglobin may thus be produced. This may cause instability or insolubility of the abnormal hemoglobin, fall in its oxygen-affinity, rise in its P_{50} and change in its oxygen dissociation curve. Hemolysis, anemia or polycythemia may also result.

Sickle cell anemia and *sickle cell trait* (sickleemia) result from the inheritance of a mutant gene for the β chain of hemoglobin in the Mendelian fashion. The β -globin gene suffers in such cases from a point mutation replacing a single amino acid residue in the β chain by an abnormal amino acid. The homozygous individual inherits the abnormal gene from both parents and suffers from the full-blown disease called sickle cell anemia. Such a patient carries only the abnormal hemoglobin HbS instead of HbA in all the erythrocytes. HbS contains normal α chains, but its β chains have nonpolar valine in place of polar glutamate as the 6th amino acid residue from the N-terminal end. This abnormal valine residue on the surface of the β chains causes the interlinking of deoxygenated HbS molecules to form long, insoluble, fibrous polymers which precipitate as crystals in erythrocytes. The latter are consequently changed into highly fragile crescentic (sickle-shaped) cells which are easily hemolyzed, producing severe anemia. The condition is aggravated at low P_{O_2} because deoxygenated HbS polymerizes more easily to sickle the red cells. The homozygous patient dies early of anemia.

On the contrary, the heterozygous individual inherits the abnormal gene from only one parent and the normal gene from the other, and suffers from sickle cell trait with far less severe symptoms. Such a patient carries HbS amounting to only half of the total

circulating hemoglobin, the rest being normal HbA. The heterozygous patient is less susceptible to one type of malaria because the sickle cell gene confers resistance against malaria. This has caused the persistence and spread of the sickle cell gene in malaria-infested countries.

HbS occurs frequently among Western Indian tribals, many African races and American colored population.

Thalassemias

Thalassemias are found frequently among Kutchi-Lohanas and Sindhis in India. There is a reduced production (but no structural abnormality) of a particular type of peptide chain of globin due to a point mutation, deletion or rearrangement in the DNA in and around the gene for that peptide chain. There may simultaneously occur a compensatory rise in the synthesis of other peptide chains of globin, which replace the repressed peptide chain in globin molecules. Abnormal hemoglobins consequently result.

In α -thalassemia, synthesis of α chain is reduced or stopped due to deletions in the cluster of the α -globin gene on chromosome 16. Hemoglobin A is consequently poor or absent in erythrocytes. HbA₂ is also abnormally low. Abnormal hemoglobins, deficient in α chains, are produced and circulated. For example, hemoglobin H (β_4) made of four β chains, appears in erythrocytes in the adult stage while hemoglobin Barts (γ_4) occurs in erythrocytes of the fetus or neonatal infants. Abnormal hemoglobins with only β , γ or δ chains have altered P_{50} and oxygen-affinity, and are unaffected by the Bohr effect of high P_{CO_2} . Hemoglobin Barts causes edema and severe fatal anemia.

In β -thalassemia, the rate of β chain translation is reduced and the hemoglobins deficient in β chains, such as HbA₂ ($\alpha_2\delta_2$) and HbF ($\alpha_2\gamma_2$), rise in the patient's blood. HbA is poor or absent in erythrocytes due to

the lack of β chains. Failure of translation of the β chain may result from different types of lesions in and around the β -globin gene cluster on chromosome 11. A deletion or a rearrangement of a piece of DNA within the β -globin gene may affect the production of mRNA coding for the β chain. Translation of the β chain may also be stopped by either a frameshift in the genetic code or a change of a codon to a nonsense codon in the mRNA for the β chain, both resulting from point mutations in and around the β -globin gene. Point mutations may also affect either the regulation of transcription of the β -globin gene into mRNA or the processing of the mRNA, stopping the translation of the β chain. Often high amounts of an abnormal hemoglobin called *hemoglobin C* (HbC) occur in β -thalassemia patients (*HbC- β -thalassemia*). HbC carries normal α chains but abnormal β chains with lysine replacing the glutamate residue at the 6th position of the chain.

Synthesis of heme

Porphyrins are synthesized in many aerobic cells such as hepatocytes and developing erythrocytes. Adult man forms about 7 g of hemoglobin in 24 hours. Using isotopically labelled acetate and glycine, it has been shown that all 4 nitrogens and as many as 8 carbons of the protoporphyrin III ring come from glycine, the remaining 26 carbons come from acetate, and the α -C of glycine is the source of all four methenyl-carbons interlinking the pyrrole rings, but the carboxyl-C of glycine never enters the porphyrin molecule.

1. Synthesis of δ -aminolevulinic acid :

δ -Aminolevulinic acid (ALA or AmLev) is synthesized in mitochondria from glycine and succinyl-CoA by a mitochondrial enzyme, δ -aminolevulinic acid synthase (ALA synthase or AmLev synthase). The enzyme requires pyridoxal phosphate as its prosthetic group. In presence of Mg^{2+} , glycine first combines with the pyridoxal phosphate bound to the

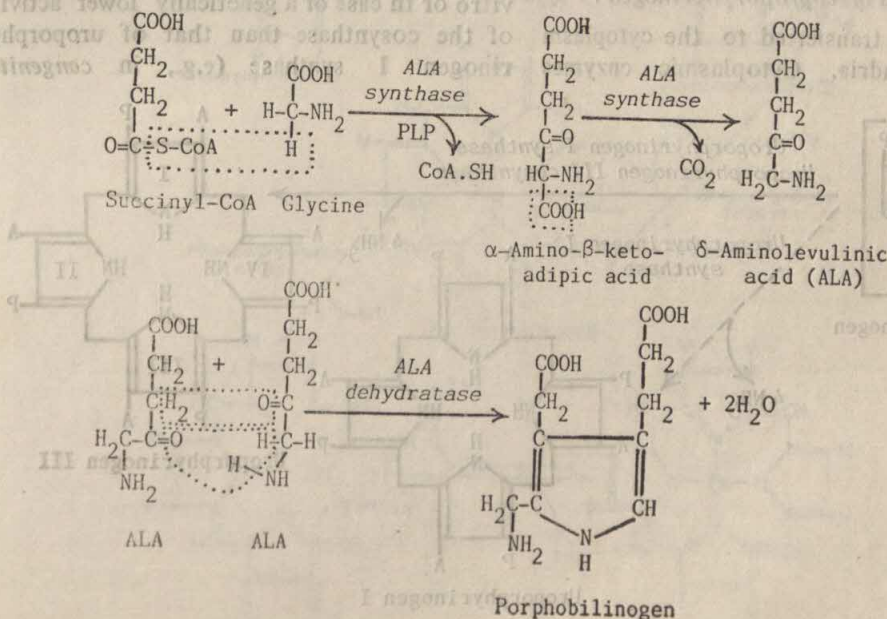


Fig. 9 31. Synthesis of porphobilinogen from succinyl-CoA and glycine.

apoenzyme. This results in the formation of a Schiff base between glycine and the enzyme-bound pyridoxal phosphate. ALA synthase next catalyzes the condensation of the enzyme-bound glycine with succinyl-CoA so that the α -carbon of glycine gets linked to the carbonyl-C of succinate, replacing coenzyme A which is released (Fig. 9.31). This results in the formation of α -amino- β -ketoadipic acid which exists as a ternary complex with pyridoxal phosphate and the enzyme. Next, ALA synthase decarboxylates α -amino- β -ketoadipate to δ -aminolevulinate and releases CO_2 and ALA. As both pyridoxal phosphate and coenzyme A are required for the synthesis of ALA by ALA synthase, deficiency of pyridoxine or pantothenate reduces heme synthesis to produce anemia. ALA synthase is a repressible as well as allosteric enzyme, and catalyzes the rate-limiting step for porphyrin synthesis. Of the activities of all the enzymes of this pathway, that of ALA synthase is the lowest in normal tissues.

2. Synthesis of coproporphyrinogen:

ALA is then transferred to the cytoplasm from mitochondria. Cytoplasmic enzymes

catalyze the conversion of ALA to coproporphyrinogen III in three steps.

(a) Two molecules of ALA are condensed by a Zn^{2+} -containing lysine enzyme, called *ALA dehydratase* or δ -aminolevulinase, to produce porphobilinogen. ALA dehydratase is an oligomeric and regulated enzyme, subject to the feed-back inhibitory effect of heme. The reaction catalyzed by ALA dehydratase proceeds through an intermediate step of an enzyme-bound Schiff base formed by the binding of the γ -keto group of ALA with the ϵ -amino group of a lysine residue of ALA dehydratase. Two molecules of water are released during the reaction (Fig. 9.31).

(b) Four molecules of porphobilinogen are next condensed together by the combined actions of *uroporphyrinogen I synthase* (porphobilinogen deaminase) and *uroporphyrinogen III cosynthase*, producing a molecule of uroporphyrinogen III with the liberation of 4 molecules of ammonia. In the presence of both the enzymes in vivo, uroporphyrinogen III is almost the sole product of this condensation; but in absence of the cosynthase in vitro or in case of a genetically lower activity of the cosynthase than that of uroporphyrinogen I synthase (e.g., in *congenital*

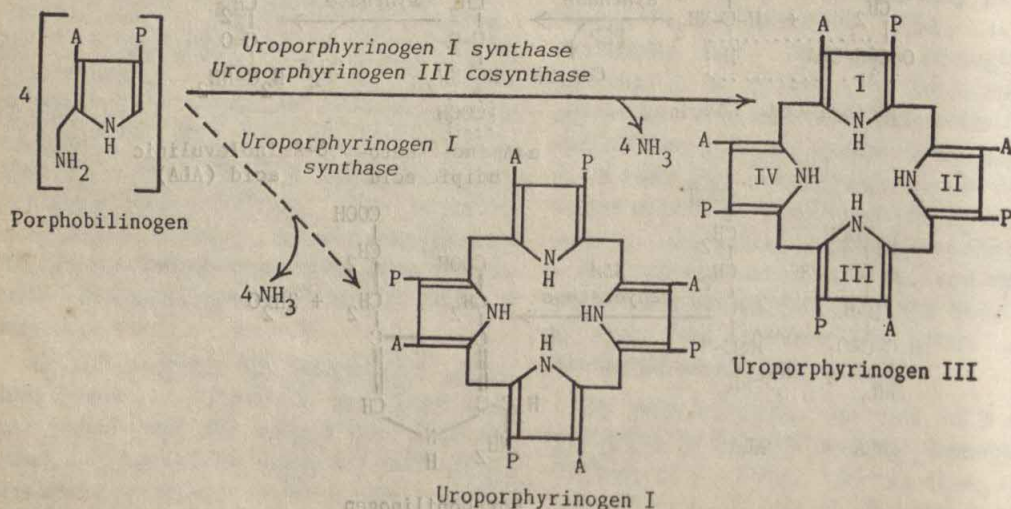


Fig. 9.32. Synthesis of uroporphyrinogens from porphobilinogen.

erythropoietic porphyria), the latter enzyme produces mainly uroporphyrinogen I from porphobilinogen (Fig. 9.32).

(c) Uroporphyrinogen III is next decarboxylated to coproporphyrinogen III by *uroporphyrinogen decarboxylase*. The enzyme decarboxylates each of the four acetate sidechains of uroporphyrinogen III, changing them to methyl groups in coproporphyrinogen III (Fig. 9.33). In case any uroporphyrinogen I has been formed in the earlier step, the decarboxylase similarly decarboxylates it to coproporphyrinogen I; this happens in congenital erythropoietic porphyria.

On exposure to light, uroporphyrinogens and coproporphyrinogens are oxidized to

corresponding uroporphyrins and coproporphyrins respectively. Unlike the colorless porphyrinogens, the porphyrins are colored substances.

3. Formation of heme :

Coproporphyrinogen III is next translocated into mitochondria from the cytoplasm. It is then converted to heme through several steps inside the mitochondria.

(a) Two of the four propionate sidechains of coproporphyrinogen III are first oxidized and decarboxylated to form vinyl sidechains under the action of *coproporphyrinogen oxidase*. This changes coproporphyrinogen III to protoporphyrinogen III (Fig. 9.33). The

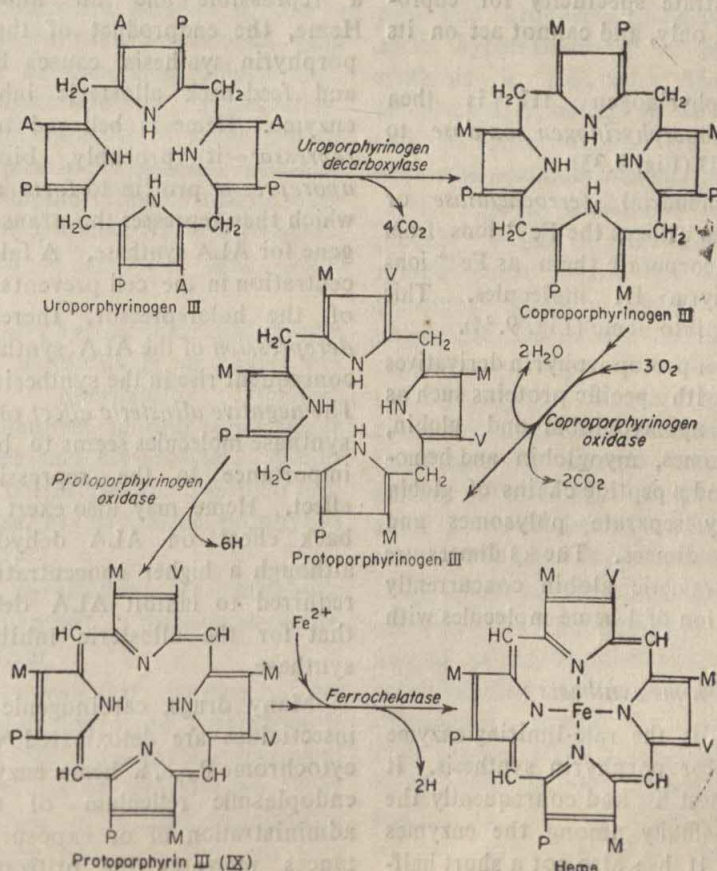


Fig. 9.33. Synthesis of heme from uroporphyrinogen.

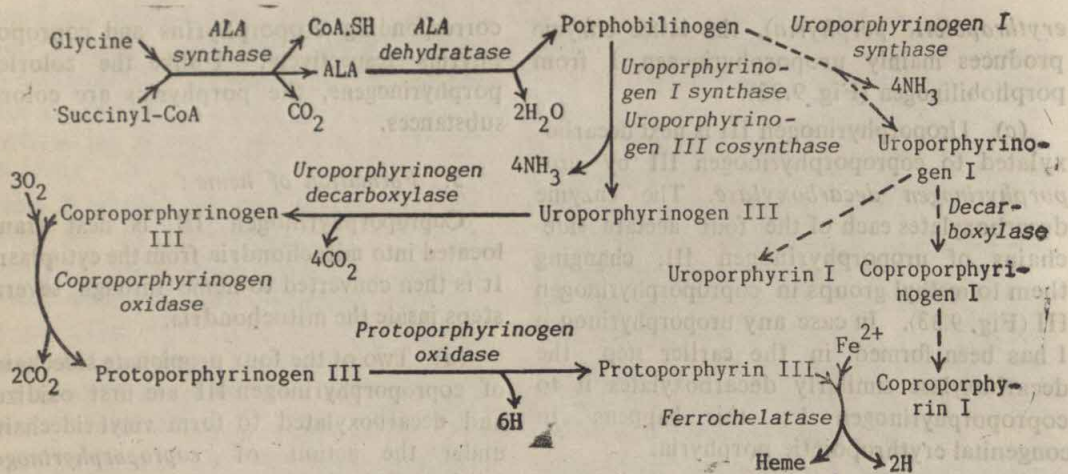


Fig. 9.34. Summary of heme synthesis.

enzyme has substrate specificity for coproporphyrinogen III only, and cannot act on its I isomer.

(b) Protoporphyrinogen III is then oxidized by *protoporphyrinogen oxidase* to protoporphyrin III (Fig. 9.33).

(c) A mitochondrial *ferrochelatase* or *heme synthase* then utilizes the Fe³⁺ ions from transferrin to incorporate them as Fe²⁺ ions into protoporphyrin III molecules. This changes the latter into heme (Fig. 9.34).

Heme and other protoporphyrin derivatives are conjugated with specific proteins such as apocytochromes, apomyoglobin and globin, to form cytochromes, myoglobin and hemoglobin. The α and β peptide chains of globin are translated by separate polysomes and condensed into $\alpha\beta$ dimers. The $\alpha\beta$ dimers are changed into tetrameric globin concurrently with the conjugation of 4 heme molecules with the peptides.

Regulation of heme synthesis :

ALA synthase, is the rate-limiting enzyme of the pathway for porphyrin synthesis. It possesses the highest K_m and consequently the lowest substrate-affinity among the enzymes of the pathway. It has also got a short half-life of about an hour. ALA synthase is both

a repressible and an allosteric enzyme. Heme, the endproduct of the pathway for porphyrin synthesis, causes both repression and feed-back allosteric inhibition of the enzyme. Heme is believed to act as a *corepressor*—it probably binds with an *aporepressor* protein to form a *holorepressor* which then represses the transcription of the gene for ALA synthase. A fall in heme concentration in the cell prevents the formation of the holorepressor, thereby causing a *derepression* of the ALA synthase gene and a consequent rise in the synthesis of the enzyme. The *negative allosteric effect* of heme on ALA synthase molecules seems to be of secondary importance to the repression-derepression effect. Heme may also exert a negative feed-back effect on ALA dehydratase activity although a higher concentration of heme is required to inhibit ALA dehydratase than that for the allosteric inhibition of ALA synthase.

Many drugs, carcinogenic substances and insecticides are detoxicated with the help of cytochrome P₄₅₀, a heme enzyme of smooth endoplasmic reticulum of the liver. So, administration of or exposure to such substances increases the utilization of heme for cytochrome P₄₅₀ synthesis, consequently

reduces intracellular heme concentration and leads to the derepression of ALA synthase and a rise in heme synthesis. Steroids accentuate the derepression of ALA synthase caused by the drugs, carcinogens and insecticides. Chelated iron may facilitate the induction of ALA synthase while hematin administration prevents the derepression of the latter.

Porphyrias

These diseases are produced by defects in porphyrin synthesis and are characterized by high blood and urinary levels of porphyrins and their precursors.

Acquired porphyrias may be produced in otherwise normal individuals by hepatic damages in hepatic cirrhosis, infective hepatitis and obstructive jaundice or due to exposure to drugs and toxic compounds such as tolbutamide, sulfonamides, chloroquine, barbiturates, phenobarbital, griseofulvin, hexachlorobenzene and salts of heavy metals like lead. Porphyrin synthesis becomes deranged due to the inhibition of one or more enzymes like ALA synthase, ALA dehydratase, uroporphyrinogen I synthase and heme synthase.

In *inherited porphyrias*, congenital defects affect porphyrin synthesis in all tissues, but the metabolic abnormality becomes predominantly manifest in only some tissues such as the liver and erythrocytes. Accordingly, inherited porphyrias are classified into hepatic porphyrias, erythropoietic porphyrias and protoporphyria.

(a) *Hepatic porphyrias*: The metabolic defects become manifest in these cases predominantly in the liver.

In *acute intermittent porphyria*, the synthesis of uroporphyrinogen I synthase is reduced to about 50% due to an autosomal dominant defect of its structural gene. This results in a failure of further metabolism of porphobilinogen and its high urinary elimination. Because of a fall in heme

synthesis, ALA synthase is derepressed to cause overproduction and a consequently high urinary level of δ -aminolevulinate. On exposure of the urine to light and air, porphobilinogen is spontaneously changed to colored porphyrins and porphobilin, turning the urine dark red. Abdominal pain and mental derangement are seen, but there is no cutaneous hypersensitivity to light.

In *hereditary coproporphyria*, there is a reduction in the conversion of coproporphyrinogen III to protoporphyrinogen III due to an autosomal dominant deficiency of coproporphyrinogen oxidase. This causes accumulation as well as high fecal and urinary elimination of coproporphyrinogen III. High tissue levels of coproporphyrinogens and uroporphyrinogens lead to cutaneous hypersensitivity to light. As heme synthesis is reduced, ALA synthase is derepressed, resulting in the overproduction and high urinary elimination of δ -aminolevulinate and porphobilinogen also.

In *porphyria cutanea tarda*, there is probably a partial deficiency of uroporphyrinogen decarboxylase due apparently to an autosomal dominant defect. Thus, uroporphyrinogen III cannot be decarboxylated to coproporphyrinogen III. This results in high urinary uroporphyrinogen III, production and urinary elimination of abnormal uroporphyrinogen I, hepatic accumulation and urinary elimination of uroporphyrins III and I produced from the uroporphyrinogens, and acute cutaneous photosensitivity.

In *porphyria variegata*, there is a partial deficiency of protoporphyrinogen oxidase due to an autosomal dominant defect. The resulting fall in the metabolism of protoporphyrinogen causes its accumulation and high urinary elimination. The reduction in heme synthesis derepresses ALA synthase, leading to the overproduction and high urinary elimination of δ -aminolevulinate, porphobilinogen, coproporphyrins and

uroporphyrins, cutaneous hypersensitivity to light, and colored and fluorescent urine.

(b) *Congenital erythropoietic porphyria*: This metabolic defect affects predominantly the erythroid cells in the red bone marrow. There is an autosomal recessively inherited deficiency of uroporphyrinogen III cosynthase causing the uroporphyrinogen I synthase activity far exceed that of the cosynthase. This leads to an excessive production of uroporphyrinogen I and coproporphyrinogen I in the red bone marrow, their accumulation in skin, erythroid cells and erythrocytes, their high elimination in the urine, extreme cutaneous photosensitivity, red and fluorescent urine, and a red fluorescence of teeth. δ -Aminolevulinate and porphobilinogen are produced in excess and eliminated in the urine because of a derepression of ALA synthase owing to a fall in heme synthesis.

(c) *Protoporphyrria or erythropoietic protoporphyria*: This results from an auto-

somal dominant deficiency of ferrochelatase in all tissues although the metabolic defects are mainly manifest in erythroid cells and liver. Erythroid cells, plasma and feces contain high amounts of protoporphyrin III. Acute urticaria is produced in sunlight due to cutaneous photosensitivity.

Catabolism of heme

Macrophages of spleen, liver and bone marrow phagocytize old erythrocytes at the average rate of about 2.5×10^9 erythrocytes in 24 hours in a normal human adult and catabolize about 6-7 g of hemoglobin present in the degraded erythrocytes. Heme and hemoglobin released from the disintegrated erythrocytes, are transported in the plasma in combination with large globulins called hemopexin and haptoglobulin respectively, to avoid their urinary elimination. Subsequently, these complexes are removed from circulation by hepatocytes and macrophages

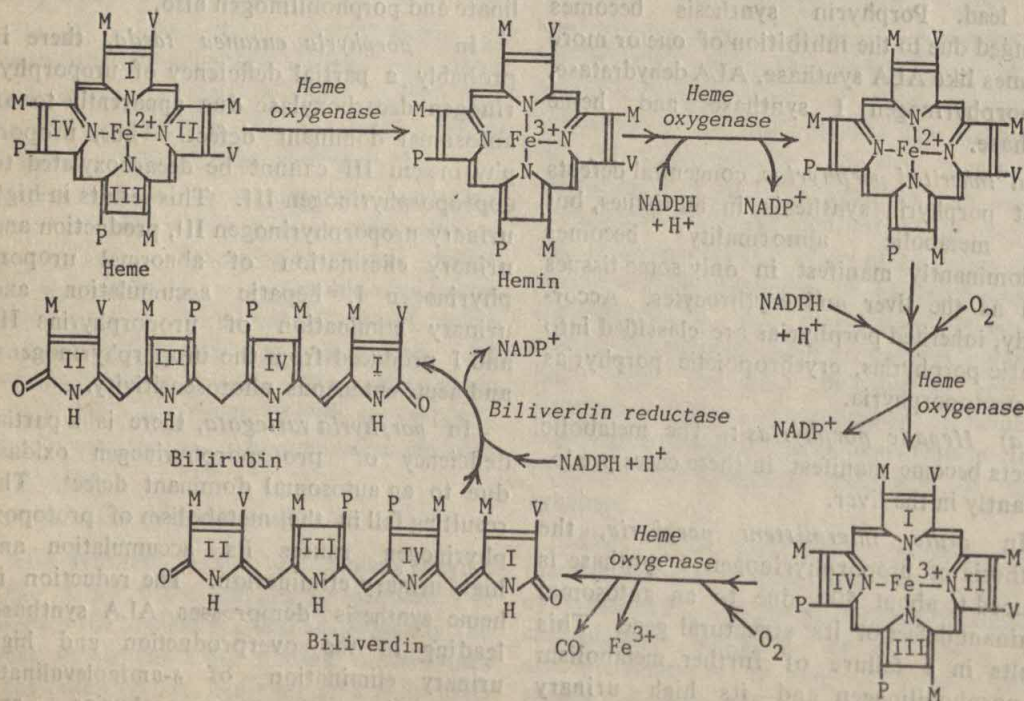


Fig. 9.35. Catabolism of heme to bilirubin.

for further degradation. The globin part of the hemoglobin molecule is split off and reutilized.

Heme is catabolized in the smooth endoplasmic reticulum of macrophages with the help of a microsomal enzyme system called *heme oxygenase* which acts in cooperation with the microsomal electron transport systems. The Fe^{2+} ion of heme is oxidized to Fe^{3+} changing heme to *hemin* (Fig. 9.35). The latter may probably bind with albumin to form methemalbumin. Its Fe^{3+} ion is again reduced to Fe^{2+} by NADPH. The methenyl bridge-C between pyrrole rings I and II of the porphyrin is next hydroxylated with molecular O_2 utilizing NADPH as the electron-donor in the process. With the help of molecular O_2 and the catalytic action of heme itself, the Fe^{2+} ion of the molecule is re-oxidized to Fe^{3+} ion. With further oxidation, the Fe^{3+} ion is released from the molecule to enter the iron pool for storage and re-utilization in heme synthesis. Simultaneously or closely following the release of Fe^{3+} from the molecule, the hydroxylated methenyl bridge-C between pyrroles I and II is released as CO, opening the tetrapyrrole structure between pyrroles I and II and producing an open-chain green pigment called *biliverdin IXa*. The latter is reduced to a yellow pigment called *bilirubin IXa* by *biliverdin reductase* and NADPH; in this reaction, the methenyl bridge between pyrroles III and IV is reduced to a methylene bridge. In this way, each gram of hemoglobin is catabolized into 35 mg of bilirubin so that more than 200-250 mg of bilirubin is produced from about 6-7 g of hemoglobin every 24 hours. Birds and amphibians lack the enzyme biliverdin reductase and excrete the green pigment biliverdin instead of bilirubin as the major pigment in their bile.

Bilirubin is released from macrophages into the blood. It is only sparingly soluble in water. Upto 25 mg of bilirubin are

carried per decilitre of blood in a tight combination with a high-affinity site of serum albumin; some more bilirubin may be carried in a loose combination with a low-affinity site of albumin. Hepatocytes take up bilirubin from the bilirubin-albumin complexes of plasma flowing in the liver sinusoids. The uptake of bilirubin by the liver cells is carried out by facilitated diffusion mediated by a carrier system of the hepatocyte membrane. A downward concentration gradient has to be maintained between the plasma and the hepatocyte interior for the continuation of the facilitated diffusion of bilirubin. This is normally maintained by the subsequent metabolism of bilirubin, once inside the hepatocyte.

After the uptake of bilirubin from the plasma, hepatocytes conjugate it with D-glucuronic acid to make bilirubin water-soluble. For this, a microsomal enzyme called *bilirubin-UDP glucuronyl transferase* first transfers a glucuronyl group from UDP-glucuronic acid to a propionic acid sidechain of bilirubin IXa, forming bilirubin monoglucuronide and UDP in the smooth endoplasmic reticulum (Fig. 9.36).

Bilirubin monoglucuronide may be further conjugated with a second molecule of glucuronic acid. This is frequently accomplished by a second *glucuronyl transferase* of the hepatocyte plasma membrane bordering the bile canaliculi. The enzyme transfers a glucuronyl group from UDP-glucuronic acid to the remaining propionic acid sidechain of bilirubin monoglucuronide, changing the latter to bilirubin diglucuronide. Alternatively, *bilirubin monoglucuronide dismutase* may transfer a glucuronyl group from one molecule of bilirubin monoglucuronide to another, changing them to bilirubin and bilirubin diglucuronide respectively (Fig. 9.36).

Some bilirubin monoglucuronide may be conjugated with an aldose with the help of a

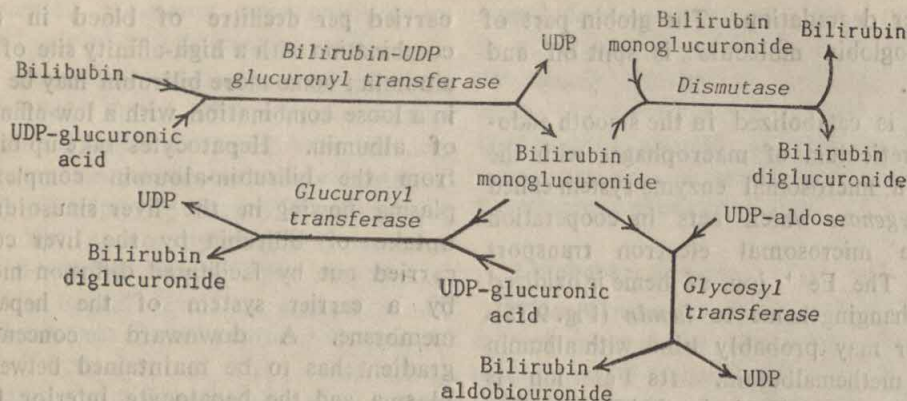


Fig. 9.36. Detoxication of bilirubin by conjugation.

glycosyl transferase to form bilirubin aldobionuride. Small amounts of bilirubin may also be conjugated with sulfuric acid in the liver. Normally, more than 97% of the bilirubin excreted in bile consists of conjugated bilirubin. The latter is actively secreted by hepatocytes into the bile canaliculi against a steep concentration gradient. This active secretion and the preceding conjugation serve as an integrated unit for the hepatic metabolism of bilirubin while the active secretory process plays the rate-limiting role for this metabolic pathway.

Bilirubin, both free and conjugated, may be re-oxidized to a minor extent in the gallbladder to yield some biliverdin. But in avian bile, biliverdin is the principal bile pigment because avian macrophages lack biliverdin reductase for reducing biliverdin to bilirubin.

Bilirubin glucuronides excreted in bile are hydrolyzed by bacterial β -glucuronidases in the intestine to yield free bilirubin which is reduced by anaerobic putrefying bacteria of intestine to colorless tetrapyrroles called *urobilinogens* (Fig. 9.37) such as L-urobilinogen (stercobilinogen). Most of the urobilinogens are oxidized to orange-yellow urobilins (stercobilins) by colonic bacteria while some of the urobilinogens are degraded by bacteria to

dipyrroles. All these products are mostly excreted in the feces normally. The feces turn darker after excretion because some fecal urobilinogens which have hitherto escaped bacterial oxidation, get oxidized to

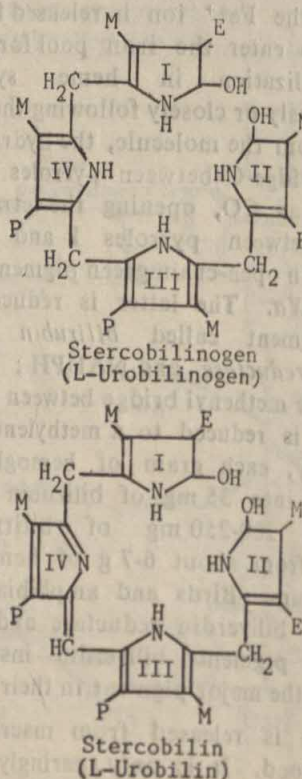


Fig. 9.37. Stercobilinogen and stercobilin.

colored urobilins on exposure to air. On the other hand, small amounts of urobilinogens are absorbed from the distal ileum and colon and subsequently excreted either in the urine or in the bile. Urinary urobilinogens may be oxidized to urobilin and mesobiliviolin on exposure to air.

Compared to as much as 250 mg of bile pigments and their derivatives eliminated in the feces and urine in 24 hours, only about 1.2 mg of coproporphyrin III and very little coproporphyrin I and uroporphyrins are normally excreted in 24 hours.

Crigler-Najjar syndromes

These rare diseases are caused by congenital defects of the bilirubin-conjugating system in the liver. The defects are inherited as autosomal recessive disorders. Type I Crigler-Najjar syndrome results from the genetic deficiency of bilirubin-UDP glucuronyl transferase which normally converts bilirubin to bilirubin monoglucuronide in the liver. Consequently, free or unconjugated bilirubin ('indirect-reacting' bilirubin of Van den Bergh test) rises in the serum and usually exceeds 20 mg dl⁻¹. Bile contains free bilirubin only and urine has a high free bilirubin content. The patient suffers from a severe congenital jaundice which damages the corpus striatum and other cerebral ganglia and causes death within 15 months of birth usually. Type II Crigler-Najjar syndrome probably results from a genetic deficiency of the glucuronyl transferase which normally converts bilirubin monoglucuronide to bilirubin diglucuronide in the liver. Free bilirubin rises in the serum, but usually does not exceed 20 mg dl⁻¹. The patient suffers from a milder congenital jaundice which may not be fatal. Bile contains some bilirubin monoglucuronide, unlike the type I disorder.

Gilbert's disease

This rare disease shows a number of heterogeneous disorders including a benign

congenital jaundice with a mild rise in free or unconjugated bilirubin in the serum. This genetic defect is probably inherited as an autosomal dominant disorder and consists of deficiencies in the hepatic uptake of free bilirubin from blood plasma and in the activities of the hepatic bilirubin-UDP glucuronyl transferase.

9.14 HYDROGEN ION CONCENTRATION

Protolysis of water

Water dissociates to a small but definite extent to give H⁺ and OH⁻ ions (*dissociation or protolysis of water*). This protolysis increases with the rise in temperature. The liberated protons (H⁺) immediately join H₂O molecules to form hydronium ions (H₃O⁺); even bigger ions like H₅O₄⁺ may arise from several associated H₂O molecules.

Due to the protolysis of water, all aqueous solutions contain some H⁺ as H₃O⁺ ions, and some OH⁻ in hydrated forms. The *hydrogen ion concentration* [H⁺] of an aqueous solution is the concentration of hydrated H⁺ ions present mainly as H₃O⁺. Conventionally, however, simple H⁺ is often used to represent H₃O⁺. From the law of mass action, at equilibrium for the reaction $\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$,

$$\frac{[\text{H}^+][\text{OH}^-]}{[\text{H}_2\text{O}]} = K_a$$

where K_a is the *acid dissociation constant* of water at the given temperature and the bracketed terms represent the molar concentrations (mol litre⁻¹) of the respective substances at equilibrium. Because very few molecules ordinarily undergo protolysis in a mass of water, the mass of undissociated water, viz., [H₂O], is practically constant. Thus,

$$[\text{H}^+][\text{OH}^-] = K_a [\text{H}_2\text{O}] = K_w$$

where K_w is the *ion product or dissociation constant of water* at a given temperature. As

each H_2O molecule dissociates into one H^+ and one OH^- , for pure water

$$[H^+][OH^-] = [H^+]^2 = K_w \text{ or } [H^+] = \sqrt{K_w}$$

At ordinary room temperature ($25^\circ C$), $K_w = 1.008 \times 10^{-14} \approx 10^{-14}$. So, for pure water and all neutral aqueous solutions at $25^\circ C$,

$$[H^+] = \sqrt{K_w} = \sqrt{10^{-14}} = 10^{-7} \text{ mol litre}^{-1}$$

As K_w is a constant, any rise or fall in $[H^+]$ of an aqueous solution is accompanied by a reverse change in its $[OH^-]$.

pH

pH is the negative logarithm of the H^+ concentration to the base 10 when the H^+ concentration is expressed in mol litre^{-1} . Stated differently, it is the logarithm of the reciprocal of H^+ concentration to the base 10; pH may also be defined as the index of the exponential term, obtained by writing the molar concentration of H^+ as a power of 10, omitting the negative sign of the term.

$$pH = -\log [H^+] = \log \frac{1}{[H^+]}$$

Thus, for pure water or neutral aqueous solutions at room temperature,

$$pH = -\log [H^+] = -\log [10^{-7}] = 7$$

Sorensen's pH scale is a logarithmic scale ranging from 0 to 14 for dilute aqueous solutions like biological fluids. pH 7 is considered the neutral pH. If an acid is added to water, protons donated by the acid raise the $[H^+]$ of the solution above $1 \times 10^{-7} \text{ mol litre}^{-1}$; if, say, $[H^+]$ now equals $2.54 \times 10^{-5} \text{ mol litre}^{-1}$,

$$\begin{aligned} pH &= -\log [2.54 \times 10^{-5}] \\ &= -\log 2.54 - \log 10^{-5} = 4.60 \end{aligned}$$

Thus, a pH lower than 7 indicates $[H^+]$ higher than $10^{-7} \text{ mol litre}^{-1}$; the greater the acidity, the lower is the pH. If a base is added to water, some of the protons donated by water are accepted by the base, lowering the $[H^+]$ below $10^{-7} \text{ mol litre}^{-1}$; if, say, $[H^+]$ now amounts to $1.63 \times 10^{-10} \text{ mol litre}^{-1}$,

$$\begin{aligned} pH &= -\log [1.63 \times 10^{-10}] \\ &= -\log 1.63 - \log 10^{-10} = 9.79 \end{aligned}$$

Thus, a pH higher than 7 indicates $[H^+]$ lower than $10^{-7} \text{ mol litre}^{-1}$; the greater the alkalinity, the higher is the pH. The rise or fall of pH by 1 signifies a tenfold fall or rise, respectively, in the H^+ concentration.

TABLE 9.10. H^+ concentrations (mol/litre) and pHs of biological fluids.

Biological fluids	pH	H^+ concentration
Aqueous humor	7.3–7.4	5.01×10^{-8} – 3.98×10^{-8}
Bile	6.9–8.6	1.26×10^{-7} – 2.51×10^{-9}
Cerebrospinal fluid	7.3–7.4	5.01×10^{-8} – 3.98×10^{-8}
Gastric juice	0.9–3.0	1.26×10^{-1} – 1.00×10^{-3}
Intestinal juice	6.4–9.1	3.98×10^{-7} – 7.94×10^{-10}
Intracellular fluids	6.0–7.1	1.00×10^{-6} – 7.94×10^{-8}
Pancreatic juice	7.1–8.4	7.94×10^{-9} – 3.98×10^{-9}
Plasma, tissue fluid	7.4	3.98×10^{-8}
Saliva	5.9–7.2	1.26×10^{-6} – 6.31×10^{-8}
Sweat	3.8–7.5	1.58×10^{-4} – 3.16×10^{-8}
Tear	5.6–8.2	2.51×10^{-6} – 6.31×10^{-9}
Urine	4.8–8.0	1.58×10^{-5} – 1.00×10^{-8}

Ionization exponent of water (pK_w) is the negative logarithm of K_w to the base 10 and equals the sum of pH and pOH.

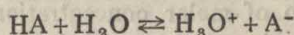
$$pK_w = -\log K_w = -\log ([H^+] \times [OH^-]) \\ = pH + pOH$$

pK_w is a constant at a given temperature. For a dilute neutral aqueous solution at room temperature, $pK_w = pH + pOH = 7 + 7 = 14$.

Acids, bases and salts

According to the *proton transfer theory* of Bronsted and Lowry, acids are substances which donate protons (H^+) to increase the H^+ concentration of their solutions, while bases accept protons to lower the H^+ concentration.

The anion, liberated by the ionization of an acid, behaves as a base and is called the *conjugate base* of the acid, because it can accept a proton (H^+) by the reverse reaction to yield the original acid. In a dilute aqueous solution, any acid HA ionizes to give its conjugate base A^- .



$$\therefore K_{eq} = \frac{[H_3O^+][A^-]}{[H_2O][HA]} = \frac{[H^+][A^-]}{[H_2O][HA]}$$

because H_3O^+ and H^+ have practically identical concentrations. (The bracketed terms represent molar concentrations). Again, because the mass of unaffected water is so vast as to remain in practically constant inspite of the reaction,

$$\frac{[H^+][A^-]}{[HA]} = K_{eq} [H_2O] = K_a$$

where K_a is a temperature-dependent ionization constant called the *acid dissociation constant*. Its values are more conveniently expressed as pK_a , called the *ionization exponent of an acid*.

$$pK_a = -\log K_a = \log (1/K_a)$$

The stronger the acid, the greater is its ionization and so, the higher is its K_a and the lower its pK_a . A strong acid like HCl possesses a high K_a of the order 10^{-3} or higher. A weak

acid like lactic acid dissociates little in solution and has a low K_a of the order 10^{-4} or lower; at equilibrium for any weak acid HA,

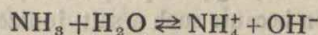
$$\frac{[H^+][A^-]}{[HA]} = K_a \quad \text{or} \quad [H^+] = K_a \frac{[HA]}{[A^-]}$$

$$\therefore -\log [H^+] = -\log K_a - \log \frac{[HA]}{[A^-]}$$

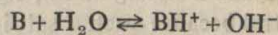
$$\text{or } pH = pK_a + \log \frac{[A^-]}{[HA]}$$

This is known as the *Henderson-Hasselbalch equation*. It follows that when $[A^-] = [HA]$, pH equals pK_a .

A base like NH_3 acts as a proton-acceptor in presence of a proton-donating solvent or acid. In an aqueous solution, H_2O donates H^+ to a base, liberating OH^- and changing the base to the corresponding cation (e.g., NH_4^+); the latter is called the *conjugate acid of that base* as it can donate H^+ by the reverse reaction.



A monoacidic base B accepts a single proton to yield the conjugate acid BH^+ in aqueous solution.



$$\therefore K_{eq} = \frac{[BH^+][OH^-]}{[H_2O][B]}$$

$$\text{or } \frac{[BH^+][OH^-]}{[B]} = K_{eq} [H_2O] = K_b$$

where the vast mass of undissociated water, viz., $[H_2O]$, is taken to remain practically constant and K_b is the temperature-dependent ionization constant called the *basic dissociation constant*. K_b is more conveniently expressed as pK_b or the *ionization exponent of a base*.

$$pK_b = -\log K_b = \log (1/K_b)$$

A *conjugate pair* consists of an acid and its conjugate base (e.g., HCl and Cl^-), or of a base and its conjugate acid (e.g., NH_3 and

NH_4^+). The stronger is one of the members of a conjugate pair, the weaker is its other member. This is explained by the reciprocal relationship between the K_a of the acid member and the K_b of the base member.

$$K_a K_b = K_w = 10^{-14}; \quad pK_a + pK_b = pK_w = 14$$

A salt ionizes in aqueous solutions to give an anion corresponding to the conjugate base of the parent acid and also a cation corresponding to the conjugate acid of the parent base. Aqueous solution of a salt is neutral, acidic or alkaline, depending on the acid strength of its cation and the basic strength of its anion. For example, NH_4Cl solution is acidic as NH_4Cl ionizes into NH_4^+ (the strong conjugate acid of the weak parent base NH_3) and Cl^- (the weak conjugate base of the strong parent acid HCl); similar other salts of strong acids and weak bases are also acidic in solution due to the same reason. Again, the aqueous solution of a salt of a weak acid and a strong alkali, e.g., Na-propionate, is alkaline in reaction because such a salt ionizes into a strong conjugate base anion (e.g., propionate ion) of the parent acid and a "neutral" cation (e.g., Na^+). The aqueous solution of a salt of a strong acid and a strong alkali, e.g., NaCl or KCl , is neutral because such a salt ionizes into a very weak conjugate base anion (e.g., Cl^-) of the strong parent acid and a "neutral" cation (e.g., Na^+ or K^+).

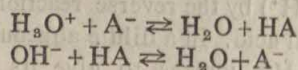
Evidently, at the endpoint of an acid-alkali titration, the pH of the titrated mixture may be acidic, neutral or alkaline according to the pH of the aqueous solution of the salt resulting from the acid-alkali interaction.

Buffers

A buffer solution resists the change of pH when acids or alkalies are added to it. Ordinarily, it contains a mixture of either a weak acid (HA) and its conjugate base (A^-), or a weak base (B) and its conjugate acid (BH^+). Because a salt (BA) ionizes to give

the conjugate base (A^-) of its parent acid, the buffer may also be constituted of a weak acid and its salt, i.e., a mixture of HA and A^- in effect. A mixture of two acid salts of a weak polybasic acid also serves as a buffer. Examples of buffer solutions include acetate buffer (acetic acid : Na-acetate), bicarbonate buffer (H_2CO_3 : NaHCO_3), phosphate buffer (KH_2PO_4 : Na_2HPO_4) and borate buffer (boric acid : Na-borate).

When a strong acid is added to a buffer solution, the H^+ ions donated by the acid (or by H_3O^+ resulting therefrom) are accepted by the base member (A^-) of the buffer to yield its acid member (HA). On the contrary, a rise in OH^- ions in the solution is neutralized by the protons donated by the acid member (HA) with the simultaneous formation of the conjugate base (A^-).



The $[\text{H}^+]$ of a buffer solution is determined by the ratio of molar concentrations of its acid and basic members and the dissociation constant (K_a) of the acid member.

$$[\text{H}^+] = K_a \frac{[\text{acid}]}{[\text{base}]} = K_a \frac{[\text{HA}]}{[\text{A}^-]}$$

So, the pH of the buffer solution is given by the *Henderson-Hasselbalch equation*.

$$\text{pH} = \text{p}K_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

Evidently, addition of a strong acid or alkali changes the ratio $[\text{A}^-]/[\text{HA}]$ of a buffer solution and so, changes the pH of the latter; but the pH change is far less than what would have occurred in absence of any buffer.

The effective range of pH for a buffer extends roughly over a pH range of ± 1 around the $\text{p}K_a$ of its acid member, i.e., within a range of 0.1 – 10.0 for the ratio $[\text{A}^-]/[\text{HA}]$.

The capacity of a buffer to resist pH changes is indicated by *Van Slyke's buffer value* (β) which is the number of moles of a

strong monoacidic base or a strong monobasic acid required to be added to 1 litre of the buffer solution to raise its pH by 1. Because the buffering capacity depends on the numbers of molecules or ions of the members of a buffer pair in the solution, a more concentrated buffer solution has a higher buffering capacity and suffers a smaller change in H^+ concentration than a diluter buffer solution, on addition of a given amount of acid or base.

Indicators

Acid-base indicators or pH indicators are weak acids or bases which change color during the pH change of their solution over a specific pH range. Each indicator exists as a conjugate pair, viz., either a weak acid and its conjugate base, or a weak base and its conjugate acid. The two members of this conjugate pair bear sharply different colors. The acid member (HIn) may change into its conjugate base (In^-) by donating protons to either water or a base, while the base member (In^-) may change into its conjugate acid (HIn) by accepting protons from either an acid or water. These changes depend on the pH of the solution. Due to

such interconversions between the two members of the buffer pair, the indicator solution changes color with the pH change. The pH of an indicator solution is given by the *Henderson-Hasselbalch equation*.

$$pH = pK_{in} + \log \frac{[In^-]}{[HIn]}$$

where the bracketed terms represent molar concentrations (mol litre^{-1} or mol dm^{-3}) of the respective substances, and pK_{in} is a constant called the *indicator exponent*. pK_{in} is the pK_a or ionization exponent of HIn and equals the pH when HIn and In^- are present in equimolecular concentrations (*half-equivalence point*). With the rise in $[H^+]$, progressively more H^+ ions are available for combining with In^- , causing a progressive fall in the ratio $[In^-]/[HIn]$; but the more alkaline the solution, the greater is the dissociation of HIn to In^- and H^+ , and consequently the higher is the ratio $[In^-]/[HIn]$. As the color of an indicator solution is a mixture of the colors due to HIn and In^- concentrations prevailing at the given pH, the solution changes color with the pH, the sharpest change occurring over ± 1 pH unit around the pK_{in} , i.e., over the 0.1–10.0 range of the ratio $[In^-]/[HIn]$; beyond this range, the color

TABLE 9.11. Some common acid-base indicators.

Indicator	Color change (acid – alkali)	pK_{in}	Effective pH range
Bromocresol green	yellow—blue	4.7	3.8—5.4
Bromocresol purple	yellow—purple	6.3	5.2—6.8
Bromophenol blue	yellow—blue	4.0	3.0—4.6
Bromothymol blue	yellow—blue	7.0	6.0—7.6
Congo red	blue—red	—	3.0—4.5
Litmus	red—blue	—	4.5—8.3
Methyl orange	red—orange	3.7	3.1—4.4
Methyl red	red—yellow	5.1	4.4—6.3
Methyl yellow	red—yellow	3.3	2.9—4.2
Neutral red	red—yellow	—	6.8—8.0
4-Nitrophenol	colorless—yellow	7.2	5.6—7.6
Phenolphthalein	colorless—red	9.6	8.3—10.9
Phenol red	yellow—red	7.9	6.8—8.4
Thymol blue	red—yellow	1.5	1.2—2.8
	yellow—purple	8.9	8.2—9.6

change is far less perceptible and too slow to be of any practical use. For the endpoint of an acid-alkali titration, the indicator should, therefore, be so chosen as to have its pK_{in} close to the pH at which the equivalence is attained between the acid and the alkali (Table 9.11); this pH is identical with that of the aqueous solution of the salt resulting from the reaction between the acid and the alkali in the titration mixture.

Indicators are used in titrimetric estimations of gastric and urinary acidities, urinary NPN, total blood fatty acid, etc. The approximate pH of a solution may be visually estimated from the color changes of a number of indicators with diverse pK_{in} —phenol red is often used for estimating pH of Ringer's and Dale's fluids while urinary pH may be estimated using methyl red, bromocresol purple and phenol red.

Spectrophotometric determination of pH: A measured amount of an indicator, having its pK_{in} close to the anticipated pH, is added to the test solution. The concentrations of In^- and HIN are then estimated in that solution by spectrophotometric measurement of light absorbancy at a specific wavelength characteristic of the indicator. pH is then computed from $[In^-]/[HIN]$ ratio, using the Henderson-Hasselbalch equation,

Biological significance of pH

1. **Tautomeric forms of purines and pyrimidines:** Purine and pyrimidine bases of nucleic acids exist in different tautomeric forms according to pH. Their specific tautomeric forms, at the body pH of nearly 7.4, are essential for the hydrogen-bonding of complementary base-pairs in DNA double-helices and RNA strands. So, pH maintains the natural three-dimensional forms of DNA and RNA molecules.

2. **Isoelectric pH:** pH influences the ionization of amino and carboxyl groups in free amino acids and in amino acid residues of

proteins. At a specific pH called the isoelectric pH of the molecule, an amino acid or a protein exists as dipolar zwitterions with minimum net charge and bearing both anionic and cationic groups. A protein or an amino acid exists as a cation below its isoelectric pH and as an anion above that pH (see pages 49, 52-54). Nucleic acids, phosphoglycerides, sphingolipids and mucopolysaccharides may also exist as zwitterions at their respective isoelectric pHs, and as cations and anions respectively below and above the isoelectric pH (see pages 32-33).

3. **Optimum pH:** By influencing ionized states of proteins, pH considerably influences the formation of ionic and hydrogen bonds which stabilize the higher orders of structure of the protein. Each protein has an *optimum pH* where it can best maintain its three-dimensional higher orders of structure befitting its biological action, and also a specific ionized state of the amino acid residues at the active site of the molecule. Any drastic change in pH may destroy the ionic and hydrogen bonds, thus changing the three-dimensional structure with denaturation and loss of biological activities. Thus, each enzyme has an optimum pH at which it acts best (see page 98). Similarly, pH influences the membrane structure by influencing the ionized states of membrane lipids.

4. **pH and Gibbs-Donnan effect:** The body pH of 7.4 is on the alkaline side of the isoelectric pHs of most body proteins which, therefore, exist as anions. Preponderance of these nondiffusible macromolecular anions on one side of semipermeable biological membranes leads to an unequal distribution of diffusible cations and anions on the two sides of the membrane for maintaining electro-neutrality on either side. Diffusible cations are more concentrated on that side of the membrane which has a higher concentration of nondiffusible protein anions while diffusible anions have a higher concentration on the

opposite side of the membrane (*Gibbs-Donnan membrane equilibrium*). pH influences such an unequal distribution of ions across semipermeable cell membranes by determining the quantity and sign of the net charge on the protein ions.

5. *Separation of proteins and amino acids*: The pH-dependence of the charged forms of proteins and amino acids is utilized in separating them from biological materials by electrophoresis and ion-exchange chromatography.

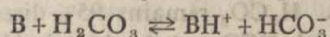
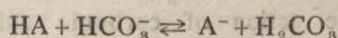
Buffers in pH regulation

Buffers minimize the pH changes in cells and extracellular fluids to maintain a constant pH in the living body.

Principal buffers of extracellular fluids include the bicarbonate buffer, the phosphate buffer and the protein buffers. Principal intracellular buffers include the phosphate buffers, the protein buffers and in erythrocytes, the hemoglobin buffer.

Bicarbonate buffer

It is the principal buffer in extracellular fluids such as the blood plasma. It comprises bicarbonate (HCO_3^-) and carbonic acid (H_2CO_3) as the base and acid members respectively. The bicarbonate buffer neutralizes stronger dietary and metabolic acids (HA), changing them to the corresponding weak conjugate bases (A^-); there is a simultaneous increase in H_2CO_3 . Stronger bases (B) are also changed to the corresponding weak conjugate acids (BH^+) with a concomitant rise in HCO_3^- .



The bicarbonate buffer has an ionization exponent (pK_a) of 6.1 and its buffering activity is consequently more effective in the pH range of 5.1 to 7.1. The normal plasma concentrations of HCO_3^- and H_2CO_3

average about 25 and 1.25 mEq litre⁻¹ respectively, which correspond to approximately 28 and 1.4 mM litre⁻¹ respectively. This gives a normal ratio of $[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$ amounting to 20 in the plasma. For the dissociation $\text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$, the *Henderson-Hasselbalch equation* indicates that the buffer ratio of 20 gives a pH of 7.4 to the solutions of bicarbonate buffer, and this is the normal blood pH.

$$\text{pH} = \text{pK}_a + \log \frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]}$$

$$\text{or pH} = 6.1 + \log 20 = 7.4$$

Whenever the bicarbonate buffer neutralizes any acid or base, the replacement of one of the members of the buffer pair by the other member changes the buffer ratio and consequently the blood pH. But the buffer ratio is immediately restored by altering either the respiratory elimination of H_2CO_3 as CO_2 or the urinary elimination of HCO_3^- .

The buffering capacity of the bicarbonate buffer suffers from two limitations in the blood. First, the molar concentrations of HCO_3^- and H_2CO_3 are relatively low in the blood; so, the addition of a given amount of acid or base to the blood tends to change its H^+ concentration considerably. Second, at the blood pH of 7.4, the bicarbonate buffer has to operate far away from its pK_a of 6.1 and beyond its effective pH range, and consequently on a portion of its buffer curve where its buffering power is poor. But these limitations are effectively countered by the following factors, making the bicarbonate buffer of prime importance in the regulation of blood pH. (i) The concentration of HCO_3^- far exceeds that of the phosphate buffer in the plasma. (ii) The bicarbonate buffer functions in close cooperation with the hemoglobin buffer of erythrocytes for buffering CO_2 . (iii) H_2CO_3 is in labile equilibrium with the dissolved CO_2 and the plasma Pco_2 ; a rise or fall in the latter causes reverse

changes in the $[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$ ratio and the pH of plasma. (iv) The $[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$ ratio can be easily regulated either by the renal adjustment of HCO_3^- elimination or by the respiratory adjustment of CO_2 elimination. Indeed, plasma HCO_3^- content is called the *alkali reserve* because of its outstanding role in buffering acids entering the blood.

The bicarbonate buffer has far less importance inside the cell because cells contain much lower amounts of HCO_3^- . The $[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$ ratio is as low as 11 in some cells like erythrocytes.

Phosphate buffer :

It is next to the bicarbonate buffer in importance in extracellular fluids other than blood. It is also of primary importance in most cells other than erythrocytes. In the blood plasma, its concentration is only about 8% of that of the bicarbonate buffer; so, its total buffering capacity is far lower than bicarbonate in the plasma. The phosphate buffer comprises dibasic phosphate (HPO_4^{2-}) and monobasic phosphate (H_2PO_4^-) as the base and acid members respectively. It has a pK_a of about 6.8 for the dissociation $\text{H}_2\text{PO}_4^- \rightleftharpoons \text{H}^+ + \text{HPO}_4^{2-}$; it is consequently more effective in the pH range of 5.8 to 7.8. So, at the blood pH of 7.4, it operates relatively close to its pK_a and well within its effective pH range and consequently on a portion of its buffer curve where its buffering capacity is high. Plasma normally has a $[\text{HPO}_4^{2-}]/[\text{H}_2\text{PO}_4^-]$ ratio of 4. The *Henderson-Hasselbalch equation* indicates that this buffer ratio corresponds to the blood pH of 7.4.

$$\text{pH} = pK_a + \log \frac{[\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]}$$

$$\text{or } \text{pH} = 6.8 + \log 4 = 7.4$$

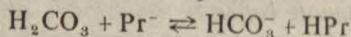
The phosphate buffer has a far higher concentration in intracellular fluids than in extracellular fluids. Moreover, the pH of intracellular fluids (6.0–6.9) is closer to the

pK_a of the phosphate buffer. These two factors endow the phosphate buffer with much higher buffering capacity inside the cells. Similar reasons make the phosphate buffer considerably effective in the urine inside the renal distal tubules and collecting ducts.

When HPO_4^{2-} buffers any acid (including some H_2CO_3), H_2PO_4^- is produced and the $[\text{HPO}_4^{2-}]/[\text{H}_2\text{PO}_4^-]$ ratio tends to change. The ratio can be restored by renal elimination of H_2PO_4^- , but not by respiratory adjustment.

Protein buffers :

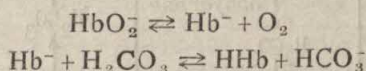
These are of considerable importance in the plasma and the intracellular fluids, but are too low in concentration in lymph, interstitial fluids and cerebrospinal fluid. Many of the proteins in the plasma are acidic proteins with their isoelectric pHs in the acidic range. So, at the blood pH of 7.4, these exist as anions to serve as conjugate bases (Pr^-) and may accept H^+ ions to form the corresponding conjugate acids (HPr). Protein buffers may even buffer some H_2CO_3 in the blood.



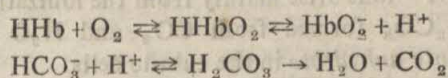
Hemoglobin buffer :

It plays the major role in buffering CO_2 inside erythrocytes, particularly due to reversible changes in the buffering capacity of hemoglobin on oxygenation and deoxygenation. Deoxyhemoglobin is a weaker acid (pK_a 8.18) and consequently possesses a much higher capacity than oxyhemoglobin (pK_a 6.62) for accepting H^+ and buffering CO_2 . On entering the erythrocytes in tissue capillaries, CO_2 combines with H_2O to form H_2CO_3 under the action of carbonic anhydrase (Fig. 9.38). H_2CO_3 remains 95% dissociated into H^+ and HCO_3^- at the blood pH of 7.4 and consequently needs immediate buffering. Side by side, oxyhemoglobin (HbO_2 or HHbO_2) has lost O_2 to form deoxyhemoglobin (Hb^- or HHb). But while HHbO_2 remains about 85% ionized as HbO_2^- at

pH 7.4, 85% of Hb^- formed by the deoxygenation of HbO_2^- remains as undissociated HHb by accepting H^+ from the ionization of H_2CO_3 . Thus, Hb^- buffers H_2CO_3 in erythrocytes.



Some of the HCO_3^- ions, formed in this buffering, diffuse out into the plasma to maintain the balance between intracellular and plasma bicarbonates. This results in a simultaneous influx of some Cl^- into erythrocytes along the electrical gradient produced by the HCO_3^- outflow (*chloride shift*). Subsequent oxygenation of HHb in lungs produces HHbO_2 which immediately ionizes into H^+ and HbO_2^- to a large extent due to its much lower pK_a . The released H^+ ions are buffered by HCO_3^- inside erythrocytes to form H_2CO_3 which is dissociated by carbonic anhydrase into H_2O and CO_2 . The latter diffuses out from erythrocytes and escapes in the alveolar air. Some HCO_3^- ions return from the plasma to erythrocytes in exchange of Cl^- ions and are in turn changed to CO_2 .



Acidosis and alkalosis

Acidosis is a fall in the $[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$ ratio of blood below 20, threatening to lower the blood pH. Hypoventilation (e.g., in asthma, respiratory paralysis or lobar pneumonia) causes CO_2 retention and raises the Pco_2 and H_2CO_3 of blood with little change in HCO_3^- . This tends to lower the $[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$ ratio and the blood pH (*respiratory acidosis*). On the other hand, excessive loss of bases (e.g., in diabetic ketosis, severe diarrhoea and nephritic acidosis) lowers the plasma HCO_3^- concentration with little change in H_2CO_3 concentration, thus lowering their ratio (*metabolic acidosis*).

Alkalosis is a rise in the $[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$ ratio of blood above 20, tending to raise the blood pH. Pulmonary hyperventilation (e.g., in thermal panting, fever, mountain sickness and voluntary hyperpnea) removes excess CO_2 from the blood and lowers the Pco_2 and the H_2CO_3 content of blood with little change in HCO_3^- , thus tending to raise the $[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$ ratio and the blood pH (*respiratory alkalosis*). On the other hand, violent vomiting, pyloric stenosis or high intake of alkaline substances may raise the plasma HCO_3^- with little change in H_2CO_3 , thereby raising their ratio (*metabolic alkalosis*). A mild metabolic alkalosis is also produced during gastric HCl secretion.

In acidosis and alkalosis, the body endeavours to restore the normal $[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$ ratio by changing the pulmonary elimination of CO_2 and/or the urinary elimination of HCO_3^- . If it succeeds to restore the normal buffer ratio, the acidosis or alkalosis is said to be *compensated*. If the buffer ratio fails to return to normal, the acidosis or alkalosis is said to be *uncompensated*.

Lungs in pH regulation

Lungs serve to normalize the $[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$ ratio and the pH of blood by changing the rate of respiratory elimination of CO_2 from the blood. A rise in alveolar ventilation lowers the alveolar Pco_2 and consequently increases the diffusion of CO_2 to the alveolar air from the dissolved state in blood. As the concentration of H_2CO_3 is in equilibrium with that of dissolved CO_2 in the blood, hyperventilation increases the $[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$ ratio simultaneously with a fall in the CO_2 concentration. Thus, a doubling of ventilation may raise the blood pH by 0.4. Hypoventilation, on the contrary, increases the blood concentration of dissolved CO_2 and consequently lowers the buffer ratio. Thus, a fall in alveolar ventilation to $\frac{1}{4}$ th the normal value may lower the blood pH by 0.46. The

pulmonary ventilation is adjusted according to the blood pH. The blood pH exerts a feed-back effect on pulmonary ventilation through the medullary respiratory centre. For example, a fall in blood pH to 7.1 raises the alveolar ventilation to almost 2.5 times the normal value while a rise in blood pH to 7.6 reduces the ventilation to almost half the normal value. Thus, acidosis and alkalosis may produce respectively hyperventilation and hypoventilation of the lungs and these in turn respectively raise and lower the $[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$ ratio to normal by respectively washing off and retaining more CO_2 . The role of lungs in the functioning of hemoglobin buffers through the oxygenation of hemoglobin, has been mentioned earlier.

Role of kidneys in pH regulation

Kidneys normally eliminate 40-80 mEq of nonvolatile acids in 24 hours and conserve bases by minimizing their urinary elimination. The pH of the glomerular filtrate is almost 7.4 as it enters the proximal tubule. But the pH of the filtrate falls progressively as the filtrate flows along the proximal and distal tubules and the collecting ducts. The filtrate pH may be lowered to about 6.9 in the proximal tubule, then to about 6.6-6.5 in the distal tubule, and ultimately to about 4.5-4.7 in the collecting ducts. That the urinary pH never falls below 4.5, practically precludes the possibility of eliminating free strong acids. The urinary pH is maintained by a cooperation between the urinary buffers and the renal ion-exchange mechanism. Major urinary buffers consist of bicarbonate and phosphate buffers which come into the urine by filtration from glomeruli. As the filtrate proceeds along the tubules, the ratio between the base member and the acid member of each urinary buffer falls progressively with a consequent fall in the urinary pH.

The renal ion-exchange mechanism includes an exchange of Na^+ and H^+ between the renal

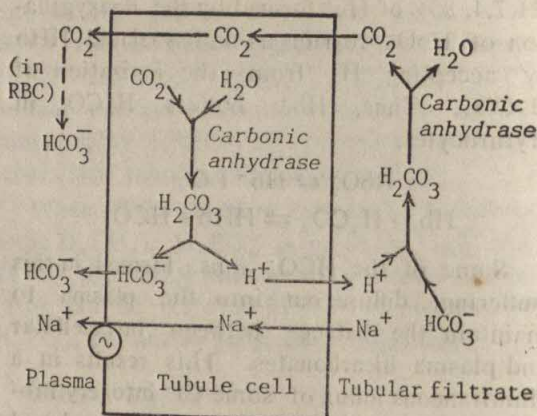
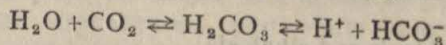


Fig. 9.38. Buffering of H^+ by HCO_3^- in renal tubules.

tubule cells and the tubular filtrate. In this process, some urinary Na^+ ions are actively reabsorbed by the tubule cells and in exchange, an equivalent amount of H^+ ions is secreted into the tubular filtrate. Of the total amount of H^+ secreted by the tubules, nearly 85% are secreted by the epithelial cells of proximal tubules and the remaining 15% by the cells of distal tubules and collecting ducts. The H^+ ions arise mainly from the ionization of H_2CO_3 formed from CO_2 and H_2O by carbonic anhydrase in the tubule cells.



While the H^+ ion is secreted in the urine, the HCO_3^- formed simultaneously is returned to blood along with the reabsorbed Na^+ ion. High metabolic rate or pulmonary hypoventilation enhances the rate of renal secretion of H^+ because the CO_2 concentration rises in the extracellular fluids in these conditions, making more CO_2 available to tubule cells for the production of H_2CO_3 . Low metabolic rate or pulmonary hyperventilation lowers the rate of H^+ secretion owing to a fall in the CO_2 concentration in extracellular fluids.

Although most of the urinary H^+ ions are secreted in the proximal tubules, the H^+ concentration in the proximal tubular lumen

does not exceed 3.2 times that in the extracellular fluid because of three reasons. (i) Much of the secreted H^+ ions are immediately buffered by HCO_3^- ions filtered from the plasma into the glomerular filtrate. This largely reduces the concentration of free H^+ ions in the tubular filtrate. (ii) The H_2CO_3 produced by such buffering in the proximal tubules is immediately removed through cleavage to CO_2 and consequently cannot play any role in lowering the pH there. (iii) The tubular filtrate continues to remain dilute in the proximal tubules and is still isotonic to plasma ($300 \text{ mOsm litre}^{-1}$). Thus, the bicarbonate-mediated buffering of H^+ ions in the proximal tubules allows neither the H^+ concentration to exceed $1.26 \times 10^{-7} \text{ mol litre}^{-1}$ nor the pH to fall below 6.9 in the proximal tubular fluid.

The secretion of H^+ continues in exchange of reabsorbed Na^+ even in the collecting ducts till the urinary pH declines to about 4.5. At this stage, the H^+ concentration in the tubular filtrate attains a maximum level of about $3.16 \times 10^{-5} \text{ mol litre}^{-1}$ which means nearly an 800 times rise above that in the extracellular fluid. H^+ ions cannot be secreted any more against such a high concentration gradient between the filtrate and the blood. This explains why the urinary pH never falls below 4.5.

The buffering of the secreted H^+ ions is, therefore, essential for continuing their secretion in the urine. This is carried out in the tubular filtrate in several ways.

(a) *Buffering by bicarbonate*: The normal average molar concentration of HCO_3^- in the glomerular filtrate amounts to about $27.9 \text{ mM litre}^{-1}$. This corresponds to an average amount of 3.49 mM of HCO_3^- being secreted in the glomerular filtrate every minute so long as the glomerular filtration rate averages 125 ml min^{-1} (inulin clearance). This rate of filtration of HCO_3^- normally falls short of the average rate of tubular secretion of H^+

by only 0.01 mM because normally about 3.50 mM of H^+ are secreted in the tubules every minute. So, most of the secreted H^+ combine with HCO_3^- in the tubular filtrate to form H_2CO_3 , leaving only a small amount of free H^+ ions to pass into the urine. The proximal tubule is the site for about 80-99% of such HCO_3^- -mediated buffering of H^+ ions depending on the rate of renal flow, blood P_{CO_2} and the HCO_3^- concentration of the extracellular fluid. The H_2CO_3 formed in this process is immediately cleaved into H_2O and CO_2 in the proximal tubular lumen by *carbonic anhydrase* occurring in the luminal plasma membrane of proximal tubule cells (Fig. 9.38). This CO_2 diffuses very readily into the proximal tubule cell and therefrom to the blood. In erythrocytes, carbonic anhydrase converts this CO_2 into H_2CO_3 which dissociates to give fresh HCO_3^- ions. Thus, the HCO_3^- ions which were filtered out in the glomerular filtrate are restored in the plasma. Some of the CO_2 diffusing into the proximal tubule cell may be converted in those cells to H_2CO_3 by carbonic anhydrase. HCO_3^- ions, formed by the dissociation of H_2CO_3 in these tubule cells, may diffuse into the blood, accompanying the reabsorbed Na^+ .

Thus, whenever H^+ ions are secreted in excess due to a fall in the blood pH, almost all the filtered HCO_3^- ions change into H_2CO_3 by combining with H^+ and are returned to the plasma as CO_2 to restore its HCO_3^- concentration. Thus, the urinary HCO_3^- is negligible so long as the urinary pH does not exceed 6. But whenever the blood pH tends to rise, much more HCO_3^- ions are filtered than the amount of H^+ ions secreted. So, some of the filtered HCO_3^- ions fail to get H^+ ions to combine and consequently fail to be returned to the plasma. This causes the urinary elimination of bicarbonate.

The buffering of the secreted H^+ ions by filtered HCO_3^- ions serves two purposes. (i) It does not allow the pH to fall below 6.9 in

the proximal tubule and consequently allows more H^+ ions to be eliminated by tubule cells into the urine. (ii) It helps to reabsorb the filtered HCO_3^- and to restore it in the blood. The tubule membrane is far less permeable to large and anionic HCO_3^- than to CO_2 . The secreted H^+ ions join HCO_3^- ions to give H_2CO_3 which readily breaks into CO_2 and the latter very readily diffuses from the tubular filtrate to the blood to form fresh HCO_3^- ions there. As the maintenance of the blood pH depends largely on the unchanged $[\text{HCO}_3^-]/[\text{H}_2\text{CO}_3]$ ratio, the kidney helps to maintain the blood pH by reabsorbing the filtered HCO_3^- and restoring it in the blood.

Although 1-20% of the HCO_3^- -mediated buffering of H^+ ions takes place in the distal tubules and collecting ducts, these tubules play minor roles in restoring HCO_3^- to the plasma because their luminal plasma membranes do not carry carbonic anhydrase.

(b) *Buffering by phosphate buffer*: Some secreted H^+ ions are buffered by the phosphate buffer, particularly in the distal tubules. HPO_4^{2-} , filtered into the glomerular filtrate, accepts the secreted H^+ to form H_2PO_4^- (Fig. 9.39). This changes the $[\text{HPO}_4^{2-}]/[\text{H}_2\text{PO}_4^-]$ ratio from 4 in the Bowman's capsule to 0.02-0.05 in the final urine; the lower

this ratio, the more acidic is the urine. But unlike H_2CO_3 , H_2PO_4^- is eliminated in the urine carrying some Na^+ ions with it and consequently causes some urinary loss of Na^+ . Moreover, the concentration of HPO_4^{2-} is far less than that of HCO_3^- in the plasma and consequently in the glomerular filtrate also; so, the total buffering power of urinary phosphate buffer is far less than that of bicarbonate buffer. However, the tubular filtrate entering the distal tubule has a pH near 6.9 which is very close to the pK_a of 6.8 of the phosphate buffer. So, at such an acidic tubular pH, the phosphate buffer operates close to its maximum buffering power. Moreover, as the urine gets progressively concentrated in the distal tubules and collecting ducts, the rise in tubular concentration of HPO_4^{2-} enhances the buffering capacity of the phosphate buffer considerably.

(c) *Buffering by ammonia*: Some H^+ ions are buffered in the distal tubule by the base NH_3 , synthesized and secreted by the tubule cells. *Glutaminase* hydrolyzes glutamine in the renal mitochondria into glutamate and NH_4^+ . This ammonia, arising from the amide group of glutamine, accounts for about $\frac{2}{3}$ rd of the total secreted ammonia. The remaining $\frac{1}{3}$ rd of the secreted ammonia comes from the cleavage of L-glutamate to α -ketoglutarate and NH_3 by mitochondrial L-glutamate dehydrogenase and NAD^+ (Fig. 9.40). In the tubular lumen, NH_3 combines with H^+ to form its conjugate acid, viz., NH_4^+ , which is excreted in the urine in association with anions like Cl^- and SO_4^{2-} left behind by the reabsorbed Na^+ . NH_4^+ behaves like a weak acid and does not dissociate much; so, its formation effectively lowers the tubular concentration of free H^+ ions, enabling further secretion of H^+ . The tubular membrane, though permeable to free NH_3 , is not permeable to NH_4^+ ions which are consequently retained in the tubular filtrate instead of diffusing back into the tubule cells (*diffusion*

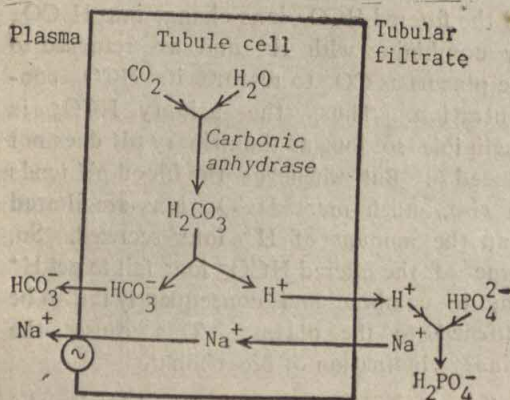


Fig. 9.39. Buffering of H^+ by HPO_4^{2-} in renal tubules.

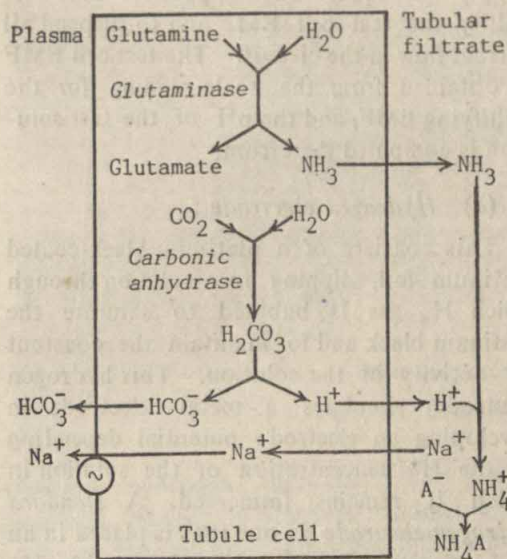


Fig. 9.40. Buffering of H^+ by NH_3 in renal tubules.

trapping). Further, conversion of NH_3 to NH_4^+ reduces the tubular concentration of free NH_3 , enabling more NH_3 to diffuse from the distal tubule cell to the tubular filtrate. So, the higher the amount of H^+ left in the filtrate after the buffering actions of HCO_3^- and HPO_4^{2-} , the greater is the rate of tubular NH_3 secretion. Thus, the secretion of NH_3 rises whenever the free H^+ concentration is high enough to lower the urinary pH below 6; the more acidic the urine, the higher is the urinary ammonia. An alkaline urine, with no free H^+ ion left for binding with NH_3 , contains little or no NH_3 . Kidneys normally excrete about 40 mEq of NH_4^+ in 24 hours. But continued elimination of highly acidic urine for several days may gradually enhance ammonia secretion by even 15 times the normal level.

(d) *Elimination of free acids*: Some H^+ ions are accepted by strong conjugate bases such as lactate, urate and oxalate anions, replacing the Na^+ reabsorbed from their salts.

Free weak acids such as lactic and uric acids result. These may be excreted without any base because they have poor ionizability and their elimination changes the urinary pH but little. For example, 50% of acetoacetate is eliminated as free acid in the urine during ketosis. But strong acids such as sulfuric and hydrochloric acids cannot be eliminated in free forms even at the lowest possible urinary pH. So, Cl^- and SO_4^{2-} are excreted in association with Na^+ , NH_4^+ and other cations.

Renal correction of alkalosis:

Alkalosis is compensated by increasing the urinary elimination of HCO_3^- so as to lower the $[HCO_3^-]/[H_2CO_3]$ ratio and the blood pH to normal. In alkalosis, the blood carries a disproportionately high amount of HCO_3^- compared to the amount of dissolved CO_2 . So, the glomerular filtrate contains far more HCO_3^- ions than the H^+ ions secreted in the tubules. Because HCO_3^- is reabsorbed as CO_2 through the formation of H_2CO_3 , tubule cells cannot reabsorb the HCO_3^- ions in excess of the tubular H^+ ions. The consequent urinary elimination of unabsorbed HCO_3^- ions causes a loss of HCO_3^- from blood and consequently lowers the buffer ratio and the pH of blood. Alkalosis also reduces the tubular secretion of NH_3 .

In respiratory alkalosis resulting from excessive respiratory elimination of CO_2 , the alkali reserve (blood HCO_3^- content) may be adjusted at subnormal levels during the renal compensation of alkalosis.

Renal correction of acidosis:

Acidosis is compensated by decreasing the urinary elimination of HCO_3^- so as to retain the HCO_3^- in the blood for raising the $[HCO_3^-]/[H_2CO_3]$ ratio and the blood pH to normal. In acidosis, the blood carries a disproportionately high amount of dissolved CO_2 compared to that of HCO_3^- . So, the tubule cells secrete far more H^+ ions than

what HCO_3^- ions are filtered from glomeruli. This enables all the filtered HCO_3^- ions to form H_2CO_3 by combining with H^+ and consequently, to get reabsorbed as CO_2 from the tubules. Thus, the urine contains no HCO_3^- while the reabsorbed HCO_3^- is restored to blood to raise the buffer ratio. Moreover, tubular secretion of NH_3 rises for buffering the H^+ ions left in the tubules after all HCO_3^- ions have been reabsorbed.

In respiratory acidosis resulting from reduced respiratory elimination of CO_2 , the alkali reserve may be adjusted above the normal level during the renal compensation.

Potentiometric determination of pH

A pH meter consists of a concentration cell (test cell), made of two half cells placed in a circuit with a potentiometer. The latter supplies an adjustable opposite EMF to balance and nullify the test cell EMF (Fig. 9.41). The half cells consist respectively of a *reference electrode* (a hydrogen or calomel electrode) and an *indicator electrode* (a glass, hydrogen or calomel electrode). The reference electrode is dipped in a standard solution of known H^+ concentration while the indicator electrode is immersed in the test solution whose pH is being determined. An EMF is generated in the test cell depending on the difference between the two solutions in H^+ concentration. The potentiometer is adjusted to

supply an equal but opposite EMF to nullify the test cell EMF and to suspend all current flow in the circuit. The test cell EMF is obtained from the scale-reading for the nullifying EMF, and the pH of the test solution is computed therefrom.

(a) Hydrogen electrode :

This consists of a platinum black-coated platinum foil, dipping in a solution through which H_2 gas is bubbled to saturate the platinum black and to maintain the constant H^+ activity of the solution. This hydrogen electrode simulates a metal electrode in developing an electrode potential depending on the H^+ concentration of the solution in which it remains immersed. A *standard hydrogen electrode* is one that is placed in an HCl solution of unit H^+ activity with H_2 bubbled at 1 atm pressure through the solution. Its electrode potential is arbitrarily taken as 0 volt for all temperatures. If a concentration cell is constituted by joining the standard hydrogen electrode as the reference electrode with an indicator electrode (say, a calomel electrode) placed in an unknown (test) solution of different H^+ activity, an EMF (E) is generated in the cell depending on the difference between the two solutions in H^+ concentration (Fig. 9.42).

$$E = \frac{RT}{nF} \log_e \frac{\text{unit } \text{H}^+ \text{ activity}}{\text{H}^+ \text{ concentration of test solution}}$$

$$= \frac{RT}{nF} \log_e \frac{1}{[\text{H}^+]} = 0.059 \text{ pH}$$

where R = molar gas constant, $T = 298 \text{ K}$ (25°C), n = valence of the ion of electrode material, and $F = 96487$ coulombs.

(b) Saturated calomel electrode :

It is a standard half cell consisting of metallic mercury over which lie successively a layer of $\text{Hg-Hg}_2\text{Cl}_2$ paste in saturated KCl solution and a layer of saturated KCl solution (Fig. 9.42). It is standardized against a

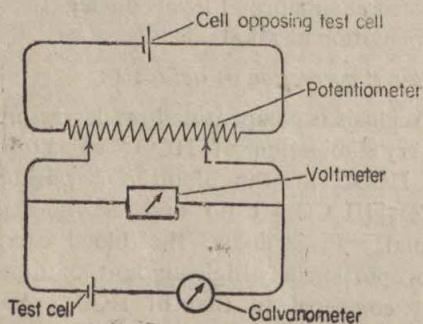


Fig. 9.41. Potentiometric measurement of pH.

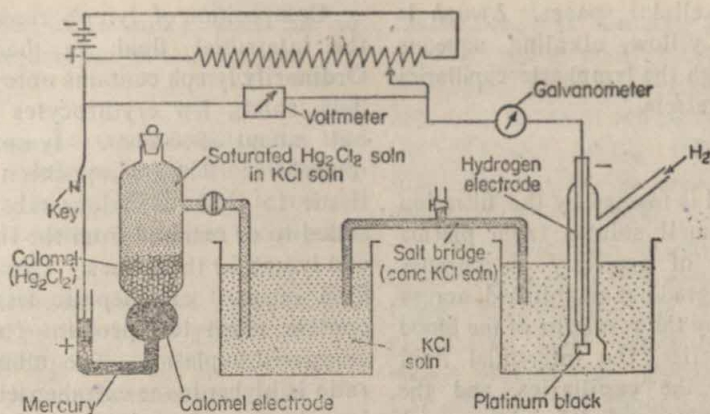


Fig. 9.42. Calomel and hydrogen electrodes.

standard hydrogen electrode. The EMF generated between a standard calomel electrode as the reference electrode and another half cell dipped as the indicator electrode in the test solution, differs by 0.244 volt from the EMF of a concentration cell with standard hydrogen electrode as the reference electrode. So, with the calomel electrode as the reference electrode at 298 K,

$$E - 0.244 = 0.059 \text{ pH, or } \text{pH} = \frac{E - 0.244}{0.059}$$

(c) *Glass electrode :*

pH meters commonly carry a standard saturated calomel electrode as the reference electrode and a glass electrode as the indicator electrode. The glass electrode measures pHs from 2 to 11 with considerable precision. It consists of a glass bulb, filled with an electrolyte solution of constant H^+ activity (0.1M). A platinum wire, plated with Ag/AgCl and having a constant electrode potential, remains immersed in this standard electrolyte solution of the glass bulb (Fig. 9.43). If the glass bulb is dipped in a test solution differing in H^+ concentration from the standard electrolyte solution inside the bulb, H^+ ions flow across the glass membrane separating the two solutions, but other

ions cannot cross that membrane. This generates a potential difference between the two sides of the glass membrane, depending on the H^+ concentration of the test solution. This electrode potential of the glass electrode can be measured with reference to the adjoined standard calomel electrode, using the nullifying EMF of a potentiometer.

9.15 INTERSTITIAL FLUID AND LYMPH

Tissue fluid or interstitial fluid is the clear, alkaline, colorless, aqueous fluid in the

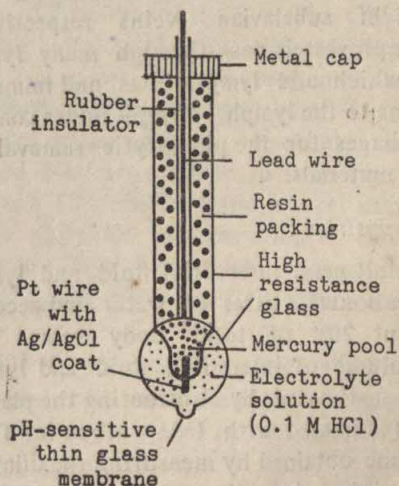


Fig. 9.43. Glass electrode.

extravascular intercellular spaces. *Lymph* is the clear, faintly yellow, alkaline, aqueous fluid flowing through the lymphatic capillaries and larger lymph vessels.

Origin and fate :

Interstitial fluid is formed by the filtration of water and small solutes from plasma through slit-pores of capillary membranes down the pressure gradient established across those membranes by the resultant of the blood pressure in capillaries, the interstitial fluid pressure outside the capillaries, and the colloid osmotic pressures of plasma and interstitial fluid.

Some interstitial fluid gets reabsorbed by diffusion and osmosis into the venous ends of capillaries. The remaining tissue fluid enters the lymphatic capillaries through minute 'flap valves' formed on their walls by the free, flap-like, overlapping edges of contiguous endothelial cells. The local compression of tissues, and the suction effect of periodic expansions of lymph capillaries are responsible for this fluid flow into the lymph capillaries.

Lymph flows through progressively bigger lymph vessels into the thoracic and right lymphatic ducts which drain into the left and right subclavian veins respectively. The lymph vessels pass through many *lymph nodes* which add lymphocytes and immunoglobulins to the lymph. Lymph nodes contain macrophages for the phagocytic removal of foreign materials.

Composition :

In adult man, interstitial fluid and lymph contain about 7-8 litres of water and account for about 20% of total body water. The total volume of interstitial fluid and lymph may be estimated by subtracting the plasma volume (estimated with I^{131} -albumin) from the volume obtained by measuring the dilution of some injected inulin or mannitol (which becomes distributed only in the ECF).

Composition of lymph resembles that of the interstitial fluid in the same tissue. Ordinarily lymph contains upto 95-96% water, 3-4% solids, few erythrocytes and platelets, but about 500-20000 lymphocytes per microlitre. The composition varies from tissue to tissue as various substances may be added to or removed from the interstitial fluid and lymph by the tissues. Lymph and tissue fluid of most extrahepatic tissues ordinarily contain much less proteins (only 0.3 - 3.5%) compared to plasma. The albumin : globulin ratio is higher in the extrahepatic lymph than in plasma. Immunoglobulins and small amounts of prothrombin and fibrinogen occur in lymph.

Due to the addition of plasma proteins synthesized by liver cells, the hepatic lymph contains 6-7% protein, and has a much higher albumin : globulin ratio than the lymph from extrahepatic tissues. Intestinal lymph is intermediate between hepatic and extrahepatic lymphs in protein concentration.

Lower concentrations of protein anions in the extrahepatic lymph lead to lower concentrations of inorganic cations and higher concentrations of inorganic anions in it than in plasma; 100 ml of extrahepatic lymph ordinarily contain about 300-310 mg Na^+ , 16-18 mg K^+ , 8-10 mg Ca^{2+} , 380-410 mg Cl^- and 5-7 mg Pi. Inorganic ion concentrations of hepatic lymph resemble those of plasma because of the higher protein concentration in that lymph.

Non-protein organic constituents include about 80-140 mg of glucose, 25-140 mg of NPN, 23-25 mg of urea, about 5 mg of amino acids, 1.4 mg of creatinine, a trace of uric acid and 270-370 mg of lipids and sterols per dl of lymph. About $\frac{1}{4}$ th of the lipids consists of free and esterified cholesterol; the remainder comprises triglycerides and phospholipids. Thoracic duct lymph has a high lipid concentration (upto 7-12%) after a high-fat diet, due to chylomicrons coming from intestinal

lymph; the latter looks milky white due to chylomicron droplets and is called *chyle*.

Functions :

1. Interstitial fluid participates in the exchange of nutrients, respiratory gases, water, inorganic ions, metabolic and secretory products and hormones, between the plasma and tissue cells.

2. Fluctuations in chemical constituents, pH and physical properties of plasma cannot immediately affect the tissue cells as they are separated from plasma by interstitial fluid.

3. Lymph transports serum albumin and fibrinogen from the liver, and immunoglobulins and lymphocytes from lymph nodes to the plasma, because these cannot enter the blood capillaries but can easily diffuse through the 'flap valves' in the wall of lymph capillaries.

4. Lymph transports lipids, sterols and lipoproteins in the form of chylomicrons from the small intestine to the blood.

5. Immunoglobulins and lymphocytes enter the lymph from lymph nodes to pass to the blood for helping in humoral immunity. Macrophages of lymph nodes participate in both innate and cellular immunity mechanisms.

6. Lymph redistributes the extracellular fluid in the body and prevents its stagnation in a particular tissue or organ.

9.16 CEREBROSPINAL FLUID

This extracellular fluid occurs in the cerebral ventricles, the subarachnoid spaces of the central nervous system and the central spinal canal.

Composition :

Cerebrospinal fluid (CSF) amounts to about 100-125 ml in adult man and is renewed 4-8 times in 24 hours. It is a clear, colorless slightly alkaline (pH 7.35-7.41), watery fluid with a low viscosity and a specific gravity of

1.003-1.008. It contains 98.2-99.3% water, 0.7-1.8% solids and usually 0-5 cells per microlitre. Inorganic ions include Na^+ (340-355 mg%), Cl^- (420-440 mg%), K^+ (10-15 mg%), Ca^{2+} (4.5-5.5 mg%), HPO_4^{2-} (1.4-3 mg%), Mg^{2+} (2.5-3.5 mg%), HCO_3^- (100-110 mg%) and SO_4^{2-} (0.4-0.7 mg%).

The glucose level (48-80 mg%) of CSF is lower than the blood glucose level because brain cells remove the CSF glucose for glycolysis. CSF contains 15-20 mg% of lactate produced by glycolysis in brain cells. CSF contains only about 0.2-0.4 mg of cholesterol, 7-8 mg of amino acids, 1.1-1.5 mg of creatinine, 10-15 mg of urea, 1-1.5 mg of uric acid, and about 30 mg of total NPN per dl. Protein content is quite low ($15-45 \text{ mg dl}^{-1}$) but rises as the fluid flows to the lumbar subarachnoid space. CSF contains no fibrinogen and is not coagulable. Albumin and globulins form 55-75% and 25-45% of the total proteins, respectively.

Origin and fate :

CSF is mainly secreted by the choroid plexus epithelium of cerebral ventricles. Na^+ is secreted actively against its concentration gradient while Cl^- , HCO_3^- and other diffusible anions diffuse passively into the CSF from plasma due to the electrostatic gradient caused by Na^+ secretion. K^+ ions may enter the CSF by diffusion from plasma; some K^+ may be actively reabsorbed in exchange of the secreted Na^+ ions. Due to such processes, Na^+ and Cl^- concentrations are somewhat higher while K^+ concentration is somewhat lower in CSF than in the plasma. Water passes osmotically from the plasma to the CSF.

CSF is absorbed mainly through the arachnoid villi into the dural venous sinuses of the brain by osmosis and diffusion.

Functions :

CSF (i) acts in the nervous system like the interstitial fluids of other tissues and mediates

between the blood and the brain to supply nutrients like glucose to brain cells and to remove metabolites like urea and lactate from the brain, (ii) helps to maintain a constant intracranial pressure and cerebral blood flow inspite of fluctuations in blood pressure, e.g., during a change from standing to lying posture, and (iii) prevents the access of many drugs, iodides, nitrates, cyanides, inulin, sucrose and sulfates to the brain as these cannot pass easily from the blood to the CSF.

9.17 AQUEOUS HUMOR

It is a transparent, colorless, alkaline (pH 7.3-7.4) and watery fluid in the anterior chamber of eye. Its specific gravity and viscosity are low. It contains about 99.2% water and 0.8% solids. Na^+ (400-420 mg%) and Cl^- (410-430 mg%) are higher in concentration in aqueous humor than in plasma; concentrations of HCO_3^- (105-115 mg%), Ca^{2+} (11-13 mg%) and K^+ (15-22 mg%) are similar in both fluids. L-Ascorbate is 10-40 times higher in aqueous humor than in plasma. Because lens and cornea remove glucose from aqueous humor for glycolysis to lactate, aqueous humor has a lower glucose concentration ($50-100 \text{ mg dl}^{-1}$) and a higher lactate concentration than the blood. Aqueous humor is moderately rich in hyaluronic acid whose polymerization is prevented by hyaluronidase secreted by ciliary body. It contains urea (35 mg dl^{-1}), other NPN and small amounts of proteins ($10-25 \text{ mg dl}^{-1}$), the latter consisting mainly of albumin and globulins.

Aqueous humor is secreted mainly by the ciliary processes at the rate of about 17 ml day $^{-1}$. Na^+ is actively secreted by the ciliary epithelium against its concentration gradient, with the help of an ATPase-dependent sodium pump; Cl^- , HCO_3^- and other diffusible anions diffuse into the aqueous humor from plasma

due to the electrical gradient established by Na^+ secretion. Water passes osmotically from plasma to aqueous humor. The fluid passes between the lens and the iris, through the pupil to the anterior chamber, and finally into the venous canal of Schlemm.

Aqueous humor supplies nutrients like glucose to avascular lens and cornea, and carries away metabolites like lactate and urea. The high L-ascorbate level of aqueous humor helps in ophthalmic tissue metabolism. The refractive index ($\mu = 1.33$) of aqueous humor helps in optical functions. Aqueous humor also maintains intraocular pressure.

9.18 VITREOUS BODY

This is a clear jelly-like gelatinous mass in the posterior chamber of eye. It has a refractive index of 1.34 and is very rich in hyaluronic acid polymers which impart a high viscosity to it. Hyaluronic acid occurs both in the free form and as protein-hyaluronate polymers (vitrein) whose fibrillar network holds an alkaline, low-protein aqueous fluid.

9.19 SYNOVIAL FLUID

It is a clear, alkaline (pH 7.3-7.5), non-coagulable, aqueous fluid of specific gravity 1.008-1.012 and occurs in freely movable joints. It contains about 96-97% water and and 3-4% solids. Solids include Na^+ (303-312 mg%), Cl^- (325-333 mg%), HCO_3^- (100-110 mg%), K^+ (13-18 mg%), glucose ($60-120 \text{ mg\%}$), proteins (0.5-3%), free and protein-bound hyaluronic acids ($15-800 \text{ mg\%}$), hydrolases like hyaluronidase, and NPN ($20-40 \text{ mg\%}$) including urea. Hyaluronic acid polymers make the fluid highly viscous, slippery and lubricating in function. Albumin and globulins constitute respectively 65-75% and 25-35% of the proteins. But the fluid contains no fibrinogen.

10. BIOLOGICAL OXIDATIONS

In the *anaerobic metabolism* of substrates such as glucose, there are some steps of oxidation; but such oxidations do not require molecular O_2 . On the contrary, the *aerobic metabolism* requires molecular O_2 for oxidations in its pathways. *Oxidoreductases* catalyze oxidations. The energy liberated by oxidations is largely captured by forming high-energy phosphate bonds.

10.1 OXIDOREDUCTASES

These enzymes oxidize their substrates either by removing *reducing equivalents* (H^+ and electrons) from the substrate or by adding oxygen to it. They may also reduce their substrates by reverse reactions. Oxidoreductases are classified according to their modes of action.

1. Oxygenases

They incorporate oxygen into their substrates to oxidize them. They account for the consumption of only minor amounts of O_2 and participate in synthetic or catabolic oxidations not concerned with energy production in the body. They have two subclasses.

(a) *Dioxygenases*: These catalyze the incorporation of both the atoms of an O_2 molecule into the substrate, often cleaving its molecular chain between the oxygen-acceptor carbons (Fig. 10.1). Examples: carotene 15,15'-dioxygenase; homogentisate 1,2-dioxygenase; tryptophan 2,3-dioxygenase.

(b) *Monooxygenases or hydroxylases*: These catalyze the incorporation of one

oxygen atom of the O_2 molecule into the substrate to form a hydroxyl group in the latter while the other oxygen atom of O_2 is reduced to H_2O by reducing equivalents (H^+ and e) from electron-donor cosubstrates or coenzymes like NADPH, tetrahydrobiopterin, L-ascorbate, cytochrome b_5 or cytochrome P_{450} (Fig. 10.2). Examples: phenylalanine hydroxylase, dopamine β -hydroxylase and kynurenine 3-hydroxylase.

Monooxygenase systems involving cytochromes b_5 and P_{450} form electron transport chains in hepatic microsomal (endoplasmic reticulum) membranes. *Microsomal cytochrome b_5 monooxygenases* catalyze hydroxylations of fatty acids leading to the desaturation of the latter. *Microsomal cytochrome P_{450}*

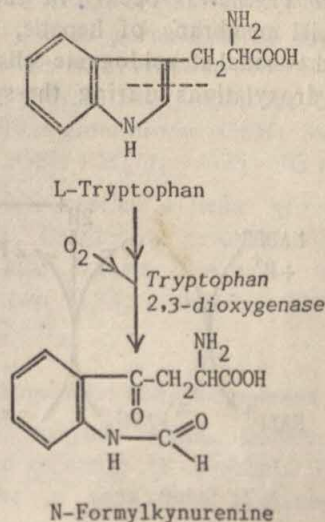


Fig. 10.1. Action of tryptophan 2,3-dioxygenase.

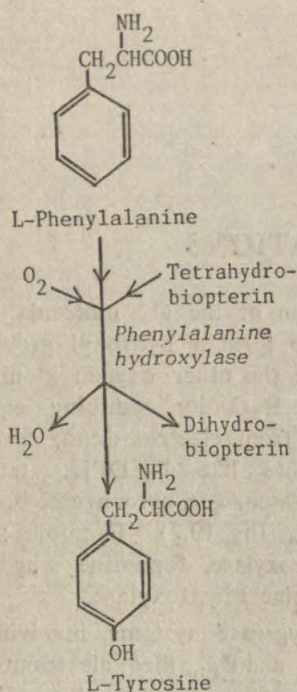


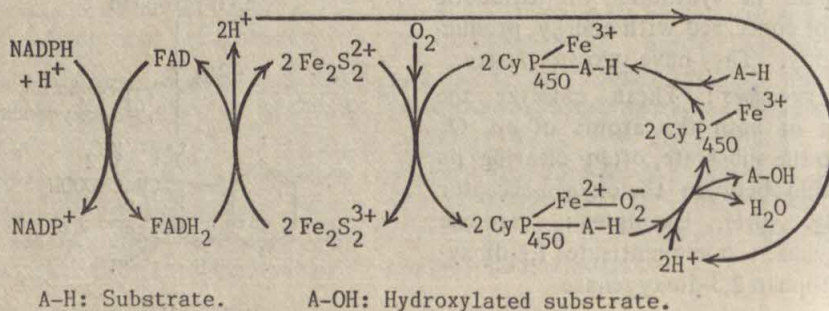
Fig. 10.2. Action of phenylalanine hydroxylase.

monooxygenases catalyze hydroxylations during the detoxication of drugs like morphine and phenobarbital. *Mitochondrial cytochrome P₄₅₀ monooxygenases* occur in the inner mitochondrial membrane of hepatic, adrenocortical and gonadal steroidogenic cells. They catalyze hydroxylations during the synthesis

of cholesterol, steroid hormones and bile acids. Cytochrome-containing monooxygenases consist of an FAD-containing flavo-protein, an iron-sulfur protein having a nonheme Fe₃S₂ centre, and either b₅ or P₄₅₀. The monooxygenase transfers reducing equivalents from NADPH or NADH to FAD, reducing the latter to FADH₂ (Fig. 10.3). FADH₂ is reoxidized by a transfer of its reducing equivalents to Fe³⁺ ions of the iron-sulfur centre, reducing them to Fe²⁺. The electrons are next transferred from the iron-sulfur protein to the Fe³⁺ of cytochrome P₄₅₀ or b₅, reducing the cytochrome. Finally, the reduced cytochrome gets reoxidized by transferring its electron to one atom of the O₂ molecule, reducing the latter to H₂O, while the other oxygen atom is incorporated into the substrate to form a hydroxyl group in it.

2. Oxidases

These oxidize the substrate by transferring reducing equivalents from it to molecular O₂ directly and produce H₂O in most cases. Oxidases are copper enzymes; some contain iron in addition. The Cu²⁺ of the enzyme receives the electron from the substrate and gets reduced to Cu⁺. The latter subsequently donates the electron to molecular O₂ and gets reoxidized to Cu²⁺. The Fe³⁺ ion, when

Fig. 10.3. Cytochrome P₄₅₀ monooxygenase action.

present, mediates in the electron transfer by accepting and donating the electron through the $\text{Fe}^{3+} \rightarrow \text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$ changes. H_2O is formed by the joining of H^+ with the O_2 which has received the electrons. Examples: cytochrome oxidase (inner mitochondrial membrane); tyrosinase (skin melanocytes); L-ascorbate oxidase (squash).

Cytochrome oxidase is a heme enzyme made of cytochromes a and a_3 , both bound to a single heptameric protein. Both the cytochromes possess heme A (Fig. 9.25) as the prosthetic group. First, the Fe^{3+} of oxidized or ferricytochrome a accepts an electron from reduced or ferrocytochrome c to oxidize the latter to ferricytochrome c, and is itself reduced to Fe^{2+} (Fig. 10.4). The Fe^{3+} of ferricytochrome a_3 then receives the electron from ferrocytochrome a to reoxidize the latter. Ferrocytochrome a_3 , produced thereby, is reoxidized to ferricytochrome a_3 by a transfer of electron from the Fe^{2+} to the Cu^{2+} of the molecule, resulting in the reduction of Cu^{2+} to Cu^+ . Finally, Cu^+ is reoxidized by donating its electron to molecular O_2 .

3. Aerobic dehydrogenases

These are flavoproteins bearing FMN or FAD in the prosthetic group. They oxidize the substrate (AH_2) by accepting two hydrogens (2H^+ and $2e$) from it on the FMN or FAD which is thereby reduced to FMNH_2 or FADH_2 (Fig. 7.11). The FMNH_2 or FADH_2 then gets reoxidized to FMN or FAD by donating the hydrogens directly to molecular O_2 , forming H_2O_2 .

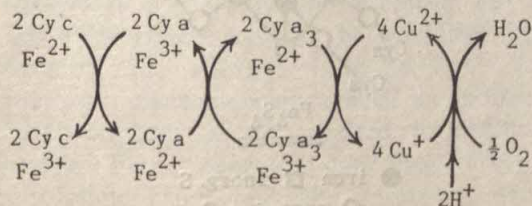
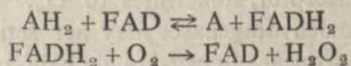


Fig. 10.4. Action of cytochrome oxidase.



But unlike oxidases, the enzymes can also donate hydrogens to artificial electron-acceptors like methylene blue. Example: L-amino acid oxidase; xanthine oxidase; urate oxidase. The last two contain iron and molybdenum which also mediate in the electron transfer.

4. Hydroperoxidases

H_2O_2 is produced by the action of aerobic dehydrogenases and also by the reduction of superoxide anions by superoxide dismutases. H_2O_2 is reduced to H_2O by hydroperoxidases such as peroxidases and catalases. Hydroperoxidases are heme enzymes and occur in hepatic and renal peroxisomes along with peroxide-producing enzymes.

Peroxidases transfer reducing equivalents (H^+ and e) to H_2O_2 from specific substrates such as glutathione, halides, L-ascorbate and ferrocytochrome c. H_2O_2 is thereby reduced to H_2O and the substrate is oxidized. Peroxidases carry protoheme as the prosthetic group. *Myeloperoxidase* of granulocytes uses Cl^- to reduce H_2O_2 : $\text{Cl}^- + \text{H}_2\text{O}_2 \rightarrow \text{OCl}^- + \text{H}_2\text{O}$. *Iodine peroxidase* of thyroid cells uses I^- in a similar reaction: $\text{I}^- + \text{H}_2\text{O}_2 \rightarrow \text{IO}^- + \text{H}_2\text{O}$. *Glutathione peroxidase* of erythrocytes utilizes glutathione (GSH) for reducing H_2O_2 : $2\text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GS-SG} + \text{H}_2\text{O}$.

Catalase carries 4 heme groups in its molecule. Besides its peroxidase-like action, it can also transfer reducing equivalents between two H_2O_2 molecules: $\text{H}_2\text{O}_2 + \text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$.

5. Anaerobic dehydrogenases

These enzymes cannot transfer electrons from the substrate to molecular O_2 directly and have to use other electron-acceptors. The electron-acceptor, reduced in the process, then transfers the electrons to somewhere

electron-acceptor, sometimes to O_2 . Several anaerobic dehydrogenases may act serially in a specific sequence in causing a chain of electron transfers from a substrate. They may thus constitute an *electron transport chain*.

(a) *Pyridine-linked dehydrogenases* :

These oxidize the substrate by transferring a *hydride ion* (H^-) from the substrate to a nicotinamide coenzyme (NAD^+ or $NADP^+$) while the second hydrogen removed from the substrate is released as free H^+ (see pages 144-149 and Table 7.2). The NAD^+ or $NADP^+$ is reduced to NADH or NADPH on accepting the hydride ion. The reduced coenzyme is next released from the dehydrogenase and is reoxidized while acting as the electron-donor coenzyme of some other dehydrogenase. The NAD^+ -dependent dehydrogenases mostly catalyze oxidations of energy-producing pathways such as glycolysis, TCA cycle and β -oxidation; the NADH produced thereby is mostly reoxidized through the donation of its hydride ion to the mitochondrial electron transport chain, yielding energy. The $NADP^+$ -dependent dehydrogenases, e.g., of the pentose phosphate pathway, produce NADPH which mainly acts as a hydride-donor for hydroxylations and reductions during fatty acid and sterol synthesis.

(b) *Flavin-linked dehydrogenases* :

Like aerobic dehydrogenases, these enzymes have FMN or FAD in the prosthetic group. In addition, some of them carry either heme or an iron-sulfur centre. They oxidize the substrate by transferring two hydrogens ($2H^+$ and $2e^-$) from the latter to either FMN or FAD of the prosthetic group. This reduces FMN or FAD to $FMNH_2$ or $FADH_2$, which then transfers reducing equivalents to an electron-acceptor other than molecular O_2 . Unlike aerobic dehydrogenases, these enzymes neither transfer electrons directly to molecular O_2 nor produce H_2O_2 . Most of these enzymes get reoxidized by transferring

reducing equivalents to coenzyme Q of the mitochondrial electron transport chain through the mediation of iron-sulfur proteins. Examples: NADH dehydrogenase (FMN-iron-sulfur-protein); cytochrome b_2 (FMN-heme-protein); succinate dehydrogenase (FAD-iron-sulfur-protein); acyl-CoA dehydrogenase (FAD-protein); dihydrolipoyl dehydrogenase (FAD-protein). These flavoprotein dehydrogenases show characteristic absorption bands at λ 370 and 450 nm when the prosthetic group is in the oxidized state (FMN or FAD), but the 450 nm band disappears when the prosthetic group is in the reduced state ($FMNH_2$ or $FADH_2$).

(c) *Iron-sulfur proteins* :

These contain non-heme iron and sulfur. They possess three types of iron-sulfur centres.

(i) *FeS centre* has a single iron atom

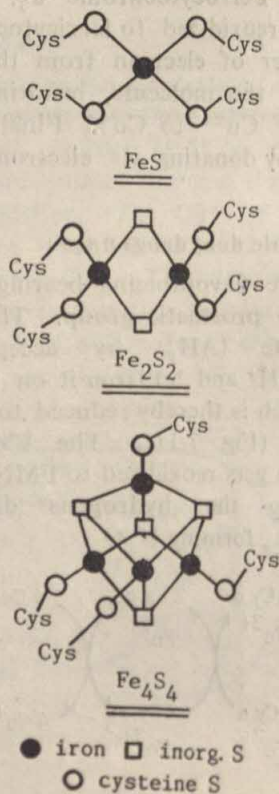


Fig. 10.5. Iron-sulfur centres.

coordinated to the sidechain SH groups of 4 cysteine residues of the apoprotein (Fig. 10.5). (ii) Fe_2S_2 centre consists of two iron atoms, two inorganic sulfides (acid-labile sulfur), and four cysteine-SH groups; each iron atom is coordinated to two of the cysteine-SH groups and also to both the inorganic sulfides. (iii) Fe_4S_4 centre consists of 4 iron atoms, 4 cysteine-SH groups and 4 inorganic sulfides; each iron atom remains coordinated to one cysteine-SH and 3 inorganic sulfides while each sulfide is coordinated to 3 iron atoms. The Fe^{3+} of the iron-sulfur protein becomes reduced to Fe^{2+} by accepting an electron either from FMNH₂ or FADH₂ of a flavoprotein oxidoreductase, or from the Fe^{2+} of reduced cytochrome b. The Fe^{2+} of the reduced iron-sulfur protein is subsequently reoxidized by the donation of its electron to an electron-acceptor like cytochrome c_1 or a ubiquinone called coenzyme Q (Fig. 10.7). The electron-affinity of iron-sulfur proteins varies largely according to their apoproteins. An iron-sulfur protein transfers only one electron at a time even if it has an Fe_2S_2 or Fe_4S_4 centre with more than one iron atom.

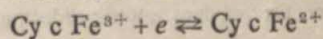
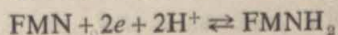
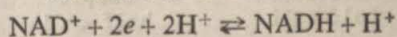
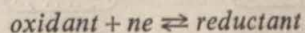
(d) Cytochromes :

These possess iron-porphyrin complexes like heme as their prosthetic groups. Oxidized forms of cytochromes are called *ferricytochromes* and contain Fe^{3+} . On accepting an electron from an electron-donor such as reduced coenzyme Q, reduced iron-sulfur protein or the reduced form of some other cytochrome, a ferricytochrome is reduced to a *ferrocytochrome* bearing Fe^{2+} . The ferrocytochrome may be reoxidized by donating the electron from its Fe^{2+} to some other electron-acceptor such as another ferricytochrome or an oxidized iron-sulfur protein. Ferricytochromes show characteristic absorption bands in the spectrum, but ferrocytochromes lack these bands. Alternate

appearance and disappearance of these bands in active tissues indicate alternate oxidation and reduction of cytochromes. Each cytochrome molecule possesses a single Fe^{3+} or Fe^{2+} ion. So, a cytochrome molecule can accept and transfer a single electron. Cytochromes a , b , c_1 and c belong to the class of anaerobic dehydrogenases. They get reoxidized by donating the electron to an electron-acceptor other than molecular O_2 . But cytochrome a_3 is auto-oxidizable and belongs to the class of oxidases. It can transfer electrons directly to molecular O_2 and forms the auto-oxidizable part of cytochrome oxidase. Cytochromes a , b , c_1 and c occur in the inner mitochondrial membrane along with cytochrome a_3 and are constituents of the mitochondrial respiratory chain. Cytochromes b_5 and P_{450} belong to microsomal and mitochondrial monooxygenase systems and are not anaerobic dehydrogenases (see page 287).

10.2 REDOX POTENTIAL

An oxidizing or reducing agent can exist in two forms: (i) the *oxidant* or oxidized form which can accept electrons from a substrate to oxidize the latter and is itself reduced thereby, and (ii) the *reductant* or reduced form which can donate its electrons to a substrate reducing the latter and getting reoxidized itself to the oxidant form. The electron-acceptor oxidant form and the electron-donor reductant form of a substance constitute a *redox couple* or *conjugate redox pair*; e.g., $NAD^+/NADH$, $FMN/FMNH_2$, fumarate/succinate and ferricytochrome/ferrocytochrome redox pairs. Where n number of electrons (e) are accepted and donated by respectively the oxidant and the reductant forms of a substance,



The electron-affinity of a substance is expressed as its *redox potential* or *oxidation-reduction potential* (E_o'). A sample half-cell is constructed by placing an electrode in a solution containing both the oxidant and the reductant forms of the substance, each at 1 M concentration. The EMF of this half-cell is called the standard redox potential (E_o), when measured against that of a standard reference half-cell, viz., a hydrogen electrode in a 1M H^+ solution (pH 0.0) in equilibrium with H_2 gas at 1 atmosphere pressure at 25°C (298 K). For biological systems, however, the standard redox potential is calculated against a H^+ ion concentration of 10^{-7} M (pH 7.0) instead of 1 M and is then expressed by the symbol E_o' instead of E_o . The two electrodes are connected to a voltmeter. The two half-cells are also joined by either an agar bridge or a KCl bridge to establish an electrical continuity (Fig. 10.6). The voltmeter reading gives the EMF which is the redox potential of the substance being investigated. If the substance has a lower electron-affinity than H_2 , its reductant form will donate electrons which will flow from the sample half-cell to the reference half-cell to join H^+ ions there, forming H_2 . So, the sample cell electrode will be negative to the reference electrode and the E_o' will also be negative. On the contrary, if the substance has a higher electron-affinity than H_2 , electrons leave H_2 and flow from

the reference half-cell to the sample half-cell and the sample cell electrode consequently becomes positive to the reference electrode, making the E_o' positive.

Evidently, redox potential (E_o') is the *electron transfer potential* under standard conditions. E_o' measures the electron-affinity and the tendency of a redox couple to donate or accept electrons under standard conditions. The more negative the E_o' of a redox couple, the weaker is its electron-affinity, the higher is the electron-donating capacity of its reductant member, and the lower is the electron-accepting ability of its oxidant. So, the reductant of a redox couple with a highly negative E_o' , is a strong reducing agent (e.g., NADPH) but its conjugate oxidant (e.g., NADP⁺) is a weak oxidizing agent. The more positive the E_o' , the higher is the electron-affinity of the redox couple, the weaker is its reductant member (e.g., ferrocyclochrom c) as a reducing agent and the stronger is its oxidant member (e.g., ferricytochrome c) as an oxidizing agent.

An oxidation-reduction always consists of a flow of electrons between two redox couples differing in electron-affinity. Electrons flow from the reductant of a redox couple with a lower electron-affinity and a lower (more negative) E_o' to the oxidant of another redox couple having a higher electron-affinity and a higher (positive or less negative) E_o' . This oxidizes the reductant of the first couple and reduces the oxidant of the second. A repetition of this event at each step makes the electrons flow successively along several redox couples constituting an electron transport chain. Electrons can be transported along such a chain because of a progressive rise in the redox potential at successive steps of the chain.

Under conditions other than the standard one, the electron transfer potential (E) of a redox couple is given by the *Nernst equation*

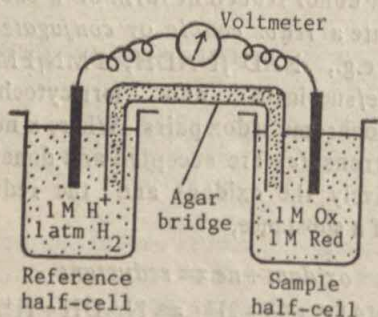


Fig. 10.6. Measurement of E_o of a redox couple. Ox: oxidant. Red: reductant.

TABLE 10.1. *Standard redox potentials at 25°C and pH 7.*

Partial reaction of redox couple	E_o' volts
Acetoacetate + $2H^+$ + $2e \rightarrow$ β -hydroxybutyrate	-0.346
α -Ketoglutarate + CO_2 + $2H^+$ + $2e \rightarrow$ isocitrate	-0.38
$NADP^+$ + $2H^+$ + $2e \rightarrow NADPH + H^+$	-0.324
NAD^+ + $2H^+$ + $2e \rightarrow NADH + H^+$	-0.320
$FMN + 2H^+ + 2e \rightarrow FMNH_2$	-0.30
Pyruvate + $2H^+$ + $2e \rightarrow$ Lactate	-0.185
Oxaloacetate + $2H^+$ + $2e \rightarrow$ Malate	-0.166
$Co\ Q + 2H^+ + 2e \rightarrow Co\ Q.H_2$	+0.10
$2Cy\ b\ Fe^{3+} + 2e \rightarrow 2Cy\ b\ Fe^{2+}$	+0.07
$2Cy\ c_1\ Fe^{3+} + 2e \rightarrow 2Cy\ c_1\ Fe^{2+}$	+0.22
$2Cy\ c\ Fe^{3+} + 2e \rightarrow 2Cy\ c\ Fe^{2+}$	+0.254
$2Cy\ a\ Fe^{3+} + 2e \rightarrow 2Cy\ a\ Fe^{2+}$	+0.29
$2Cy\ a_3\ Fe^{3+} + 2e \rightarrow 2Cy\ a_3\ Fe^{2+}$	+0.385
$\frac{1}{2}O_2 + 2H^+ + 2e \rightarrow H_2O$	+0.816

which expresses E as a function of the ratio of molar concentrations of the oxidant (ox) and its conjugate reductant (red).

$$E = E_o' + \frac{RT}{nF} \log_e \frac{[ox]}{[red]}$$

$$= E_o' + \frac{2.303 RT}{nF} \log \frac{[ox]}{[red]}$$

where R is the molar gas constant (8.314×10^7 ergs degree $^{-1}$ mol $^{-1}$), T is the absolute temperature, F is the faraday (96487 coulombs mol $^{-1}$) and n is the number of electrons transferred. At 25°C (298 K),

$$E = E_o' + \frac{0.059}{n} \log \frac{[ox]}{[red]}$$

The Nernst equation indicates that a rise in the relative concentration of the oxidant raises the electron transfer potential with a consequent increase in the electron-affinity and oxidizing capacity. It follows that the direction of electron flow between two redox couples may be changed by altering their electron transfer potentials through changes

in the $[ox]/[red]$ ratio of either or both of them. This can explain, for example, the reduction of pyruvate to lactate by NADH during anaerobic glycolysis as well as the oxidation of lactate to pyruvate by NAD^+ in aerobic conditions. Under the standard condition of 1 M concentration of both oxidant and reductant, the pyruvate/lactate redox couple has a far higher potential ($E_o' = -0.185$ V) than the $NAD^+/NADH$ redox couple ($E_o' = -0.320$ V). Anaerobic glycolysis increases both NADH and pyruvate concentrations in the cytoplasm and consequently lowers the $[ox]/[red]$ ratio of the $NAD^+/NADH$ redox couple and raises that of the pyruvate/lactate couple. These lower the electron transfer potential (E) of the $NAD^+/NADH$ couple further below that of pyruvate/lactate couple and enable NADH to donate electrons far more easily to pyruvate, reducing it to lactate. But in aerobic conditions, cytoplasmic NADH is reoxidized to NAD^+ by a transfer of its electrons to the mitochondrial NAD^+ , thereby raising the $[ox]/[red]$ ratio and the electron transfer potential of the $NAD^+/NADH$ couple; moreover, if lactate has accumulated due to a preceding phase of glycolysis, it lowers the $[ox]/[red]$ ratio and the electron transfer potential of the pyruvate/lactate couple. These raise the electron transfer potential of $NAD^+/NADH$ couple above that of pyruvate/lactate couple and enable the return of electrons to NAD^+ from lactate, reoxidizing the latter to pyruvate.

To cite another example, the oxaloacetate/malate redox couple and the $NAD^+/NADH$ redox couple possess E_o' of -0.166 and -0.320 V respectively in the standard condition; this would not allow malate to be oxidized to oxaloacetate by transferring its electrons to NAD^+ . But ordinarily the mitochondrial concentration of oxaloacetate is kept fairly lower than that of malate, lowering the $[ox]/[red]$ ratio and the electron

transfer potential of the oxaloacetate/malate couple; simultaneously, the NADH concentration is kept low by reoxidizing NADH to NAD^+ through a transfer of its electrons to the mitochondrial respiratory chain—this raises the $[\text{ox}]/[\text{red}]$ ratio and the electron transfer potential of the NAD^+/NADH couple. These factors maintain a higher (less negative) electron transfer potential of the latter than that of the oxaloacetate/malate couple in mitochondria, enabling malate to transfer its electrons to NAD^+ .

The free energy change (ΔG°) during an oxidation depends on the number (n) of electrons transferred and the difference in redox potential ($\Delta E_o'$) of the two redox systems involved. Where F is the calorie equivalent of the faraday and amounts to 23.063 kcal,

$$\Delta G^\circ = -nF\Delta E_o'$$

Whenever electrons flow from a redox couple with a lower E_o' to another with a higher E_o' , ΔG° turns out to be negative indicating a thermodynamically spontaneous oxidation with a release of energy. If two electrons flow over a $\Delta E_o'$ of +0.22 V, ΔG° amounts to -10.15 kcal and suffices for generating an ATP bond of ΔG° about -7.3 kcal.

$$\begin{aligned}\Delta G^\circ &= -nF\Delta E_o' \\ &= -2 \times 23.063 \times 0.22 \\ &= -10.15 \text{ kcal}\end{aligned}$$

10.3 ELECTRON TRANSPORT CHAINS

An *electron transport chain* consists of a properly arranged and oriented set of electron-carriers transporting electrons in a specific sequence from reduced nicotinamide coenzymes (NADH or NADPH) and flavoproteins to molecular O_2 . The chain actually consists of a series of redox couples; at each step, electrons flow from the reductant of a redox couple, having a lower (more negative or less positive) redox potential, to

the oxidant of another redox couple possessing a higher (less negative or more positive) redox potential. Different types of electron transport chains occur in the inner mitochondrial, microsomal (endoplasmic reticulum) and chloroplast membranes of eukaryotic cells, and in the plasma membrane of aerobic prokaryotic cells.

Mitochondrial respiratory chain

The inner mitochondrial membrane carries an electron transport chain called the *mitochondrial respiratory chain*. This is of prime importance as a final path through which electrons, removed from tissue substrates by oxidizing enzymes, flow to molecular O_2 . The free energy, liberated during the transit of electrons along the electron-carriers of this chain, is used in forming high-energy bonds of ATP. The respiratory chain has the following electron carriers as its main components: NADH dehydrogenase, succinate dehydrogenase, coenzyme Q, cytochromes b, c_1 , c, a and a_3 , and iron-sulfur proteins. Each of these components functions as a redox system and changes alternately to a reductant form and an oxidant form while transporting electrons.

Coenzyme Q or *ubiquinone* is the lone nonprotein component of the respiratory chain. It is a small, lipid-soluble, benzoquinone derivative having a polyisoprenoid chain of variable length; coenzyme Q_{10} , for example, has ten isoprenoid units in its side-chain (Fig. 10.7). The oxidized or quinone form of Co Q shows a characteristic absorption band at λ 280 nm, which is not shown by its reduced or hydroquinone (QH_2) form. Being nonpolar and not bound to any protein, Co Q diffuses readily in the inner membrane and makes alternate contacts with iron-sulfur proteins and cytochrome b. It is reduced to QH_2 or ubiquinol by receiving two hydrogens (2H^+ and $2e^-$) from flavoproteins, through the mediation of iron-sulfur proteins. QH_2

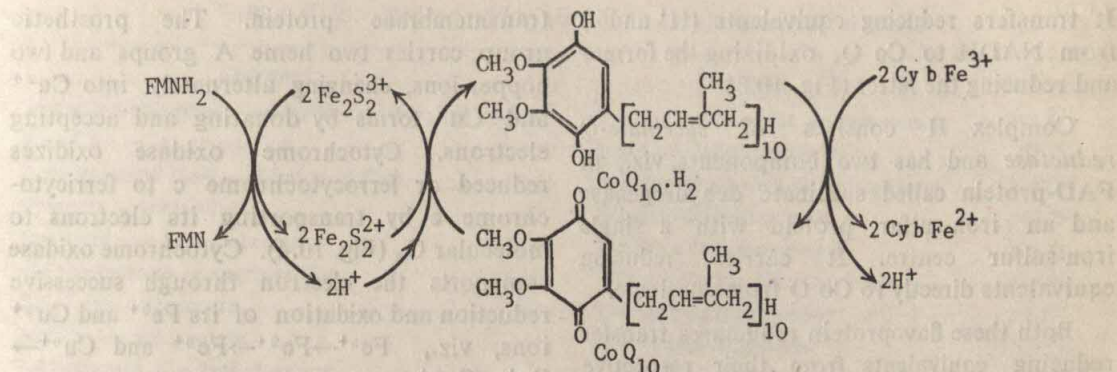


Fig. 10.7. Coenzyme Q₁₀ carrying electrons from NADH dehydrogenase (FMN-FeS-protein) to cytochrome b.

subsequently reaches cytochrome b by diffusion through the membrane and donates its electrons to cytochrome b to reduce it, thereby getting reoxidized itself to Q (Fig. 10.7). Thus, Co Q is a mobile electron-carrier between flavoproteins and cytochromes.

Except Co Q, all other components of the respiratory chain possess apoprotein groups. All of them, except cytochrome c, are integral membrane proteins. Some like NADH dehydrogenase and cytochrome oxidase (Cy a-a₃) are transmembrane integral proteins spanning the entire thickness of the inner mitochondrial membrane; some iron-sulfur proteins and the apoprotein of cytochrome c₁ are non-spanning integral proteins penetrating into only a part of the membrane thickness. On the contrary, apocytochrome c is a water-soluble peripheral protein

(MW 13 kdal) with a single peptide chain of 104 amino acid residues.

The membrane-bound components of the chain are organized in the inner membrane in specific geometrical relationships, precise asymmetric spatial orientations and an ascending order of redox potentials. These facilitate the electron flow from each component to the next, and ultimately to molecular O₂ which has a far higher redox potential than the last component (Cy a₃) of the chain. The membrane-bound components remain associated in 4 groups called *respiratory chain complexes*.

Complex I consists of *NADH-Q reductase* (NADH dehydrogenase). It is a large, oligomeric, transmembrane metalloflavoprotein (MW 850 kdal). Its prosthetic group consists of FMN and five iron-sulfur centres of both Fe₂S₂ and Fe₄S₄ types (see page 291).

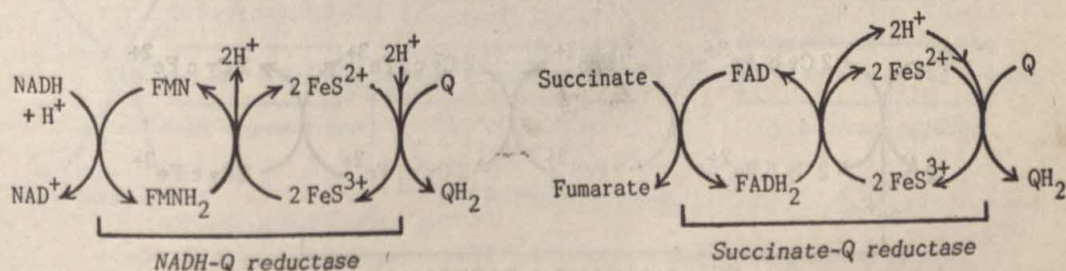


Fig. 10.8. Actions of NADH-Q reductase and succinate-Q reductase.

It transfers reducing equivalents (H^+ and e) from NADH to Co Q, oxidizing the former and reducing the latter (Fig. 10.8).

Complex II consists of *succinate-Q reductase* and has two components, viz., an FAD-protein called succinate dehydrogenase and an iron-sulfur protein with a single iron-sulfur centre. It carries reducing equivalents directly to Co Q from succinate.

Both these flavoprotein reductases transfer reducing equivalents from their respective substrates to their flavin prosthetic groups (FMN or FAD), reducing the latter. Electrons are next transferred from the reduced flavin ($FMNH_2$ or $FADH_2$) to Fe^{3+} ions of the iron-sulfur centre, reducing them to Fe^{2+} ions. Finally, the Fe^{2+} ions get reoxidized to Fe^{3+} by donating the electrons to Co Q which is consequently reduced to QH_2 .

Complex III consists of *QH_2 -cytochrome c reductase* (MW 280 kdal). It comprises cytochromes b and c_1 and an iron-sulfur protein with three iron-sulfur centres. Like cytochromes c and c_1 , cytochrome b also has heme as its prosthetic group; but unlike them, its heme group is not covalently bound to its apoprotein. Complex III transfers electrons from QH_2 to cytochrome c, the electrons flowing along its components in the following sequence: $QH_2 \rightarrow Cy\ b \rightarrow FeS \rightarrow Cy\ c_1 \rightarrow Cy\ c$ (Fig. 10.9).

Complex IV consists of *cytochrome oxidase* (MW 200 kdal). It is made of cytochromes a and a_3 , bound to a single, large, heptameric,

transmembrane protein. The prosthetic group carries two heme A groups and two copper ions, changing alternately into Cu^{2+} and Cu^+ forms by donating and accepting electrons. Cytochrome oxidase oxidizes reduced or ferrocytochrome c to ferricytochrome c by transporting its electrons to molecular O_2 (Fig. 10.4). Cytochrome oxidase transports the electron through successive reduction and oxidation of its Fe^{3+} and Cu^{2+} ions, viz., $Fe^{3+} \rightarrow Fe^{2+} \rightarrow Fe^{3+}$ and $Cu^{2+} \rightarrow Cu^+ \rightarrow Cu^{2+}$.

Each set of the four respiratory chain complexes is called a *respiratory assembly* and averages about 4×10^3 in number per μm^2 of the hepatic inner mitochondrial membrane.

Coenzyme Q and cytochrome c do not remain bound to any of the four respiratory chain complexes described above. Instead, Co Q serves to link complex I or II with complex III by carrying electrons from flavoproteins to cytochrome b while cytochrome c links complexes III and IV by transporting electrons from cytochrome c_1 to cytochrome a- a_3 (Fig. 10.10).

Most of the major cellular oxidations take place in the mitochondrial matrix during the TCA cycle, β -oxidation, aerobic oxidations of pyruvate and glycerol 3-phosphate, and oxidative deamination of glutamate (Table 10.2). Many of these oxidations are catalyzed by NAD^+ -dependent dehydrogenases because the redox potentials of these substrates are lower (more negative) than that of

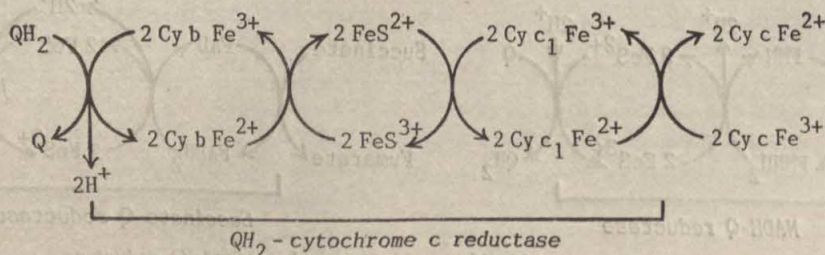


Fig. 10.9. Action of QH_2 -cytochrome c reductase.

TABLE 10.2. Major oxidations in the mitochondrial matrix.

Reactions	Enzymes	Oxidants
Glycerol 3-phosphate $\rightarrow$ dihydroxyacetone phosphate	Glycerol 3-phosphate dehydrogenase	FAD
Acyl-CoA $\rightarrow$ Δ^2 -enoyl-CoA	Acyl-CoA dehydrogenase	FAD
3-Hydroxyacyl-CoA $\rightarrow$ 3-ketoacyl-CoA	3-Hydroxyacyl-CoA dehydrogenase	NAD ⁺
Pyruvate $\rightarrow$ acetyl-CoA	Pyruvate dehydrogenase	NAD ⁺
Isocitrate $\rightarrow$ α -ketoglutarate	Isocitrate dehydrogenase	NAD ⁺
α -Ketoglutarate $\rightarrow$ succinyl-CoA	α -Ketoglutarate dehydrogenase	NAD ⁺
Succinate $\rightarrow$ fumarate	Succinate dehydrogenase	FAD
Malate $\rightarrow$ oxaloacetate	Malate dehydrogenase	NAD ⁺
L-Glutamate $\rightarrow$ α -ketoglutarate	L-Glutamate dehydrogenase	NAD ⁺

NAD⁺, at least at the prevailing [oxidant]/[reductant] ratios of the substrate and NAD⁺ in the cell (see page 293). Thus, NAD⁺ acts as the immediate electron-acceptor or oxidant for these substrates and gets reduced to NADH. The latter is reoxidized by a transfer of its hydride ion (H⁻), along with a proton (H⁺) from the medium, to the FMN of NADH-Q reductase of the respiratory chain. The reducing equivalents are then carried to molecular O₂ by other components of the chain, following the progressively rising redox potentials of successive components (Fig. 10.10).

On the contrary, succinate has a far higher redox potential (+0.031 V) than NAD⁺ (-0.320 V) and cannot donate its reducing equivalents (H⁺ and e) to NAD⁺. Instead,

succinate is oxidized by a transfer of its hydrogens to the FAD of succinate-Q reductase complex of the respiratory chain. The FADH₂ thus produced transfers its electrons to the iron-sulfur protein of the complex—redox potentials of iron-sulfur proteins vary with their apoproteins and the iron-sulfur protein of succinate-Q reductase has a higher redox potential than FADH₂. The iron-sulfur protein in turn donates the electron directly to coenzyme Q of the chain (Fig. 10.10). Succinate-Q reductase cannot transfer electrons to NADH-Q reductase because the FeS-protein of the former has a higher redox potential than the FMN/FMNH₂ redox couple of NADH-Q reductase.

Similarly, glycerol 3-phosphate and acyl-CoA are also oxidized by FAD-linked

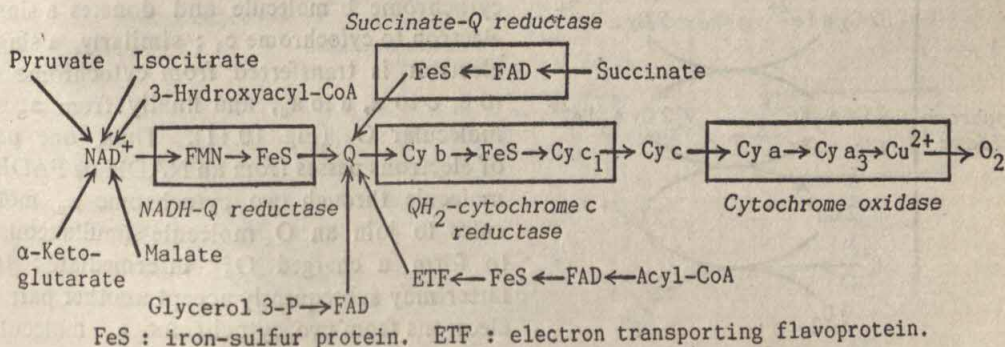


Fig. 10.10. Electron flow from different substrates through the electron transport chain.

dehydrogenases which then pass on the reducing equivalents to coenzyme Q of the respiratory chain through iron-sulfur proteins.

From cytoplasmic NADH, H^+ and electrons are carried into the mitochondrial

matrix by either malate or glycerol 3-phosphate (*mitochondrial shuttles*). In the matrix, malate donates the reducing equivalents to NAD^+ from which they are transferred to the FMN of NADH-Q reductase; from glycerol 3-phosphate, reducing equivalents pass through FAD and iron-sulfur protein to reach Co Q.

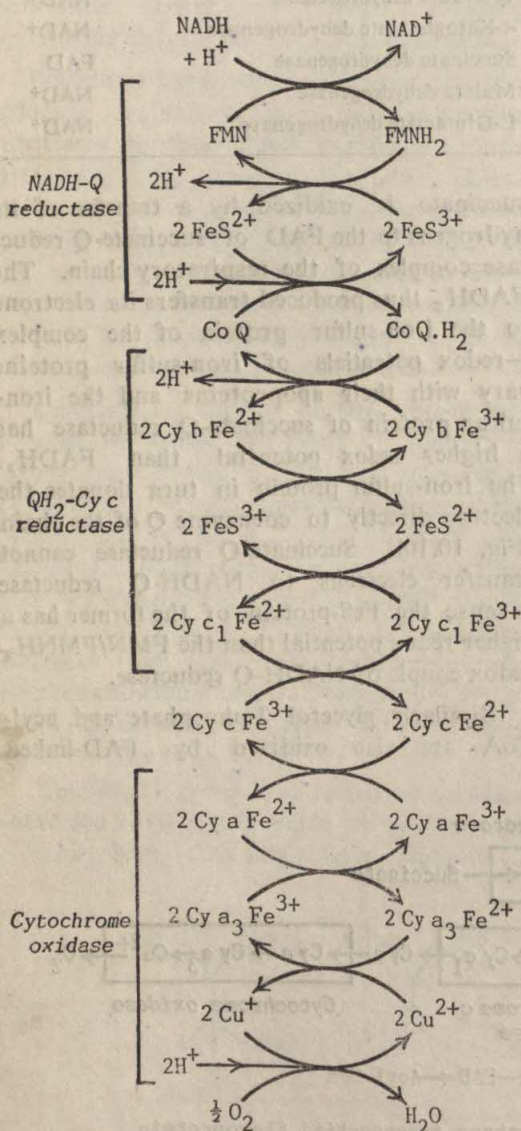


Fig. 10.11. Electron flow along the mitochondrial electron transport chain.

It may be recalled that an NAD^+ molecule accepts and transfers *one hydride ion* (H^- , i.e., one H^+ and $2e$). But each molecule of FMN, FAD or coenzyme Q accepts and donates *two hydrogens* ($2H^+$ and $2e$) at a time while each molecule of cytochromes or iron-sulfur proteins accepts and carries *only one electron* but no H^+ at all. Thus, (a) when an NADH molecule is oxidized by a transfer of its hydride ion to an FMN molecule, the latter simultaneously receives a H^+ ion from the medium to form $FMNH_2$ (Fig. 10.11). (b) Two iron-sulfur protein molecules accept two electrons from one $FMNH_2$ or $FADH_2$ molecule which simultaneously releases two protons into the medium. (c) Each coenzyme Q molecule accepts two electrons from two iron-sulfur protein molecules and simultaneously two protons from the medium, and becomes reduced to QH_2 . (d) Each QH_2 molecule in turn donates its two electrons to two cytochrome b molecules to reduce them, and simultaneously releases two protons in the medium. (e) But an iron-sulfur protein molecule receives only one electron from a cytochrome b molecule and donates a single electron to cytochrome c_1 ; similarly, a single electron is transferred from cytochrome c_1 to c, c to a, a to a_3 , and finally from a_3 to molecular O_2 (Fig. 10.11). Thus, one pair of electrons passes from an NADH or $FADH_2$ molecule through two cytochrome a_3 molecules to join an O_2 molecule simultaneously to form a charged $O_2^{\cdot -}$ intermediate; the latter may subsequently accept another pair of electrons from two cytochrome a_3 molecules and also 4 protons (H^+) from the medium to form 2 molecules of water.

TABLE 10.3. ATP-generating sites of the electron transport chain.

Enzyme	Electron flow	$\Delta E_0'$	$\Delta G^{\circ'}$
NADH-Q reductase	NADH $\rightarrow$ Co Q	+0.27 V	-12.2 kcal
QH ₂ -Cy c reductase	Cy b $\rightarrow$ Cy c ₁	+0.22 V	- 9.9 kcal
Cytochrome oxidase	Cy a $\rightarrow$ O ₂	+0.53 V	-23.8 kcal

Whenever two electrons are transferred from one redox couple to another having a redox potential higher by +0.22 V than that of the former, the free energy change ($\Delta G^{\circ'}$) is sufficiently high to drive the synthesis of a high-energy phosphate bond of ATP (see page 294). There are three such sites for ATP formation in the electron transport chain (Table 10.3).

Cross-over technique and electron transport inhibitors: Cross-over technique has been applied to find out the sequence of electron-carriers in the mitochondrial respiratory chain. Utilizing the characteristic absorption bands of the oxidized and reduced forms of each carrier, the proportion of the oxidized and reduced forms of a carrier are spectroscopically estimated after using an inhibitor for the electron transport chain. A rise in the amount of the reduced form of a carrier indicates that the latter acts at a step on the NADH or flavoprotein side of the step

blocked by the inhibitor; on the contrary, the oxidized form of the carrier rises in amount if the carrier acts on the O₂ side of the blocked step. Thus, by using inhibitors blocking different steps of the mitochondrial electron transport, the sites and sequence of action of different carriers can be revealed.

The antibiotic *piERICIDIN A*, the fish poison *rotenone* and barbiturates like *amobarbital* block the electron transport from NADH to Co Q by inhibiting the NADH-Q reductase complex. They thus block the oxidation of substrates like pyruvate, malate, isocitrate and 3-hydroxybutyrate whose electrons enter the respiratory chain through the NADH-Q reductase complex (Fig. 10.12). But they cannot prevent the oxidation of succinate whose electrons reach Co Q through the succinate-Q reductase complex instead of the NADH-Q reductase. *TTFA* inhibits the succinate-Q reductase complex by chelating the iron in its iron-sulfur component and

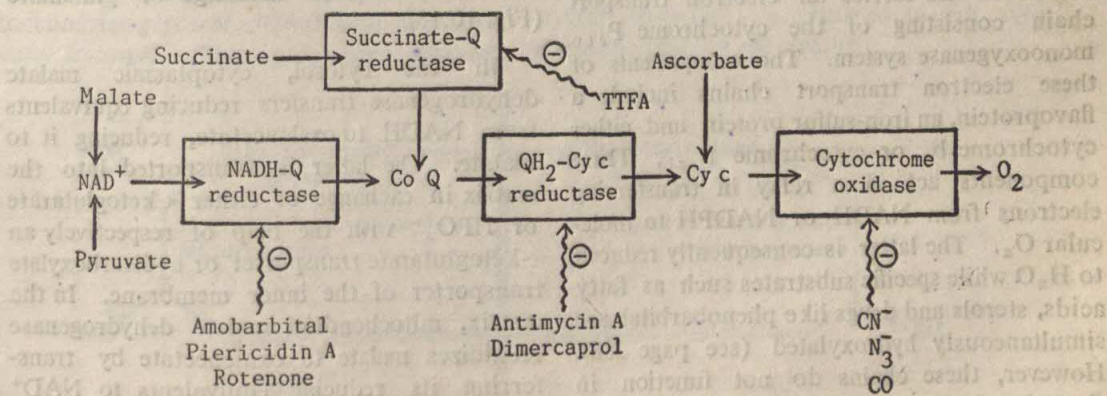


Fig. 10.12. Inhibitors blocking electron transport.

thereby blocks the oxidation of succinate. But it does not affect the oxidation of pyruvate, malate or isocitrate. *British anti-lewisite* (BAL or dimercaprol) and *antimycin A* inhibit the QH_2 -cytochrome *c* reductase complex to block the electron transport from cytochrome *b* to cytochrome c_1 of the complex. Because electrons from both NADH and FADH_2 flow through this complex, these inhibitors prevent the oxidation of pyruvate, malate, isocitrate, 3-hydroxybutyrate as well as succinate, but do not block the oxidation of ascorbate whose electrons enter the respiratory chain through cytochrome *c*, bypassing complexes I, II and III of the chain. *Cyanide* (CN^-), *azide* (N_3^-), *CO* and *H_2S* block all electron flow through the respiratory chain by inhibiting cytochrome oxidase. CN^- and N_3^- bind with the Fe^{3+} of the oxidized form of Cy *a-a*₃, while CO binds with the Fe^{2+} of reduced Cy *a-a*₃ to inhibit this step.

Other mammalian electron transport chains

Microsomal membranes have two types of electron transport chains, one consisting of the *cytochrome b_5 monooxygenase system* and the other of the *cytochrome P_{450} monooxygenase system*. The inner mitochondrial membrane also carries an electron transport chain consisting of the *cytochrome P_{450} monooxygenase system*. The components of these electron transport chains include a flavoprotein, an iron-sulfur protein and either cytochrome b_5 or cytochrome P_{450} . These components act in a relay in transferring electrons from NADH or NADPH to molecular O_2 . The latter is consequently reduced to H_2O while specific substrates such as fatty acids, sterols and drugs like phenobarbital are simultaneously hydroxylated (see page 288). However, these chains do not function in liberating free energy for ATP synthesis.

10.4 MITOCHONDRIAL SHUTTLES

The electron transport chain is so arranged in the inner mitochondrial membrane that it can accept reducing equivalents (H^+ and e^-) from the mitochondrial matrix, but not from the cytoplasm. The inner membrane being impermeable to NADH and NAD^+ , cytoplasmic NADH produced by glycolysis cannot enter the mitochondria to transfer its reducing equivalents directly to the electron transport chain. So, reducing equivalents are transferred from cytoplasmic NADH to the mitochondrial matrix by some substrates acting as carriers and shuttling between the cytoplasm and mitochondria.

Malate shuttle

This functions in cardiac muscle fibres, red muscle fibres and many other tissues. It consists of the *malate/oxaloacetate redox couple* and involves the activities of cytoplasmic and mitochondrial *malate dehydrogenase isozymes*. For running the shuttle, oxaloacetate is provided in the cytoplasm in two ways. First, ATP-citrate lyase cleaves citrate into oxaloacetate and acetyl-CoA in the cytosol. Secondly, oxaloacetate is formed in the cytosol by the transamination of aspartate brought from the mitochondrial matrix by an aspartate-glutamate transporter of the inner membrane in exchange of glutamate (Fig. 10.13).

In the cytosol, cytoplasmic malate dehydrogenase transfers reducing equivalents from NADH to oxaloacetate, reducing it to malate. The latter is transported into the matrix in exchange of either α -ketoglutarate or HPO_4^{2-} with the help of respectively an α -ketoglutarate transporter or a dicarboxylate transporter of the inner membrane. In the matrix, mitochondrial malate dehydrogenase reoxidizes malate to oxaloacetate by transferring its reducing equivalents to NAD^+ (Fig. 10.13). NADH thus produced donates

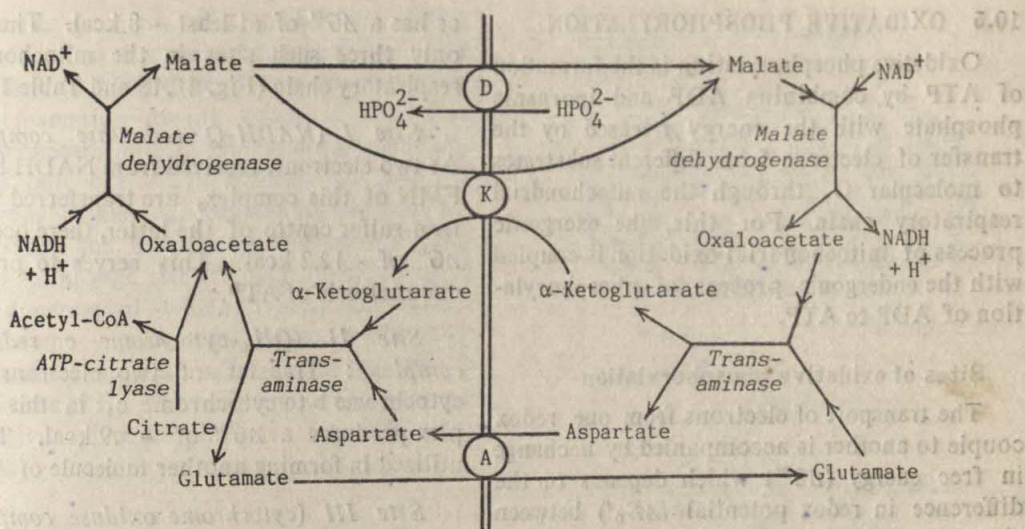


Fig. 10.13. Malate shuttle between cytosol (left) and mitochondrial matrix (right).

A : aspartate-glutamate transporter ; D : dicarboxylate transporter ;

K : α -ketoglutarate transporter.

its reducing equivalents to the FMN prosthetic group of NADH dehydrogenase of the electron transport chain.

Glycerophosphate shuttle

This functions in a limited number of tissues such as nerve cells, vertebrate white muscle fibres and insect flight muscle fibres. It consists of the *glycerol 3-phosphate/dihydroxyacetone phosphate redox pair* and involves the activities of cytoplasmic and mitochondrial *glycerol 3-phosphate dehydrogenase isozymes*. The cytoplasmic isozyme

transfers reducing equivalents from NADH to dihydroxyacetone phosphate, reducing it to glycerol 3-phosphate (Fig. 10.14). The latter is transferred into the matrix across the inner membrane and reoxidized by the mitochondrial isozyme into dihydroxyacetone phosphate by a transfer of its reducing equivalents to FAD which is thereby reduced to FADH_2 . The latter donates reducing equivalents to coenzyme Q of the electron transport chain through an iron-sulfur protein. Dihydroxyacetone phosphate is returned to the cytoplasm for running the shuttle.

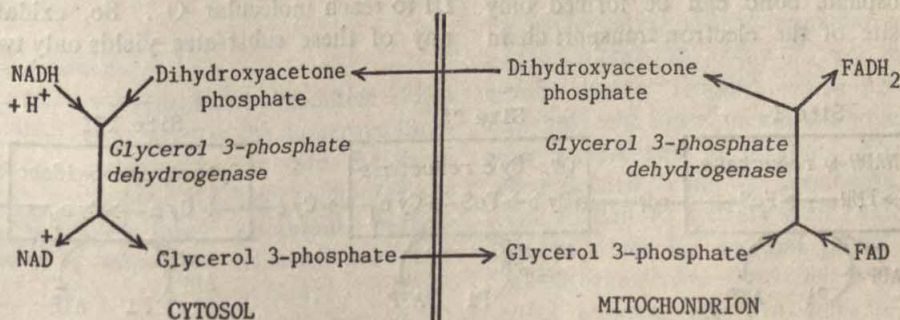


Fig. 10.14. Glycerophosphate shuttle.

10.5 OXIDATIVE PHOSPHORYLATION

Oxidative phosphorylation is the formation of ATP by combining ADP and inorganic phosphate with the energy released by the transfer of electrons from different substrates to molecular O_2 through the mitochondrial respiratory chain. For this, the exergonic process of mitochondrial oxidation is coupled with the endergonic process of phosphorylation of ADP to ATP.

Sites of oxidative phosphorylation

The transport of electrons from one redox couple to another is accompanied by a change in free energy (ΔG°) which depends on the difference in redox potential ($\Delta E_o'$) between the redox couples. Where n is the number of electrons transferred and F is the calorie equivalent of the faraday amounting to $23.06 \text{ kcal mol}^{-1}$, $\Delta G^\circ = -nF\Delta E_o'$. So, in transporting two electrons from NADH to molecular O_2 through the respiratory chain, the total free energy change amounts to about $-53 \text{ kcal mol}^{-1}$; this may be utilized in forming high-energy bonds of ATP.

$$\begin{aligned}\Delta E_o' &= [E_o' \text{ of } O_2/H_2O \text{ couple}] \\ &\quad - [E_o' \text{ of } NAD^+/NADH \text{ couple}] \\ &= +0.82 \text{ V} - (-0.32) \text{ V} = +1.14 \text{ V} \\ \therefore \Delta G^\circ &= -nF\Delta E_o' = -2 \times 23.06 \times 1.14 \\ &\approx -53 \text{ kcal mol}^{-1}\end{aligned}$$

A high-energy phosphate bond of ATP has a ΔG° of about $-7.3 \text{ kcal mol}^{-1}$. So, a high-energy phosphate bond can be formed only at such a site of the electron transport chain

as has a ΔG° of at least -8 kcal . There are only three such sites in the mitochondrial respiratory chain (Fig. 10.15 and Table 10.3).

Site I (NADH-Q reductase complex): As two electrons, received from NADH by the FMN of this complex, are transferred to the iron-sulfur centre of the latter, there occurs a ΔG° of -12.2 kcal . This serves to produce one molecule of ATP.

Site II (QH₂-cytochrome c reductase complex): Transfer of two electrons from cytochrome b to cytochrome c₁ in this complex produces a ΔG° of -9.9 kcal . This is utilized in forming another molecule of ATP.

Site III (cytochrome oxidase complex): The ΔG° amounts to -23.8 kcal for the transfer of two electrons from cytochrome a to molecular O_2 by this complex. This may be utilized to yield the third molecule of ATP.

Transport of two electrons along the respiratory chain, therefore, yields one high-energy phosphate bond at each of the three sites. Oxidation of one molecule of NADH leads to the production of three ATP molecules because the electrons donated by NADH are transported through all those three sites. But oxidation of succinate, acyl-CoA or glycerol 3-phosphate yields FADH₂ instead of NADH; electrons are transferred from FADH₂ to Co Q directly bypassing site I, and are transported only through sites II and III to reach molecular O_2 . So, oxidation of any of these substrates yields only two ATP

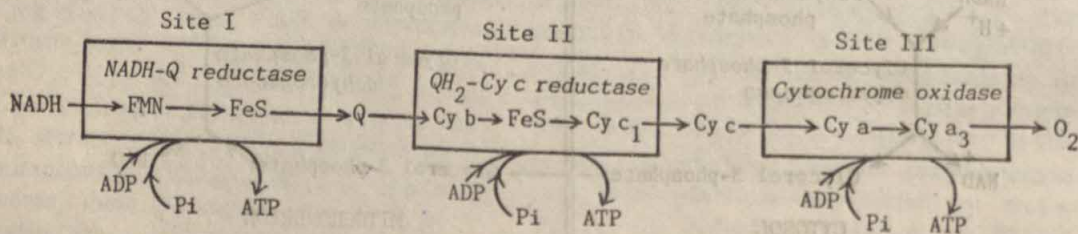


Fig. 10.15. Sites of the electron transport chain where ΔG° suffices for ATP synthesis.

molecules per pair of electrons transported. Piericidin A, rotenone and amobarbital which inhibit the NADH-Q reductase, do not inhibit ATP formation during succinate oxidation which bypasses site I. Oxidation of ascorbate yields only one ATP molecule per pair of electrons transferred, because its electrons bypass sites I and II, join cytochrome c directly and pass through site III only. Antimycin A and dimercaprol which inhibit the QH_2 -cytochrome c reductase, do not inhibit ATP formation during ascorbate oxidation which bypasses site II. CN^- , N_3^- and CO stop all oxidative phosphorylations by inhibiting site III.

P : O ratio

The *P:O ratio* or *P:2e ratio* is an index of oxidative phosphorylation. It is the number of phosphate groups esterified into ATP per atom of oxygen used or per pair of electrons transferred to oxygen.

$$\text{P:O ratio} = \frac{\text{Phosphate groups esterified}}{\text{Electron pairs transferred}}$$

All NAD^+ -dependent dehydrogenases such as those oxidizing pyruvate, malate, isocitrate and α -ketoglutarate, produce three high-energy phosphate bonds per pair of electrons transferred and consequently have a P:O ratio of 3. But the P:O ratio amounts to 2 for flavoprotein dehydrogenases oxidizing succinate, acyl-CoA or α -glycerophosphate, because their actions bypass site I of oxidative phosphorylation.

ATP synthase

Sites for ATP synthesis by oxidative phosphorylation are located in the numerous inner membrane particles projecting into the mitochondrial matrix from the inner membrane and its cristae. Each inner membrane particle consists of a knob-like spherical *headpiece* connected by a *stalk* to a *basepiece* deeply embedded in the inner membrane (Fig. 10.16). The headpiece is 8.5-9 nm in diameter. It

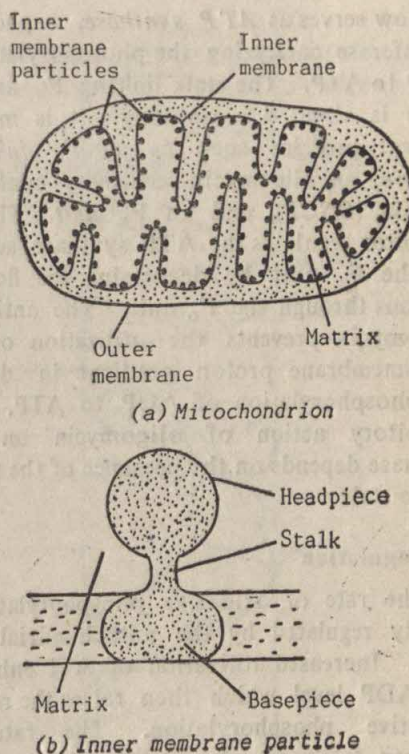


Fig. 10.16. Location and structure of mitochondrial inner membrane particles.

is made of a macromolecular oligomeric protein called the F_1 unit (MW 360 kdal). The latter is composed of α , β , γ , δ and ϵ peptide chains, some numbering more than one. The basepiece is made of an integral membrane protein called the F_0 unit which spans the entire thickness of the membrane. F_0 is composed of four hydrophobic peptide chains including a small nonpolar polypeptide called *proteolipid*. The F_0 unit contains a proton channel through which protons (H^+) can cross the inner membrane which is otherwise impermeable to protons. The F_1 unit, when isolated from the membrane, functions as an *ATPase* to hydrolyze ATP into ADP and P_i ; but when F_1 remains bound to F_0 in the mitochondrion and a proton gradient exists between the two sides of the inner membrane, the action of F_1 is reversed and

F_1 now serves as *ATP synthase*, a phosphotransferase catalyzing the phosphorylation of ADP to ATP. The stalk linking F_0 and F_1 units is about 4.5 nm long. It is made of several proteins such as an F_1 inhibitor protein, an oligomycin-sensitivity conferring protein (OSCP), and an F_0 unit. The F_1 inhibitor regulates the ATP synthase activity of the F_1 unit by decreasing the flow of protons through the F_0 unit. The antibiotic oligomycin prevents the utilization of the transmembrane proton gradient in driving the phosphorylation of ADP to ATP. This inhibitory action of oligomycin on ATP synthase depends on the presence of the OSCP in the stalk.

Regulation

The rate of oxidative phosphorylation is mainly regulated by the mitochondrial ADP level. Increased utilization of ATP enhances the ADP level which then raises the rate of oxidative phosphorylation. The rate of electron transport along the mitochondrial respiratory chain is limited by the rate of phosphorylation of ADP to ATP. So, the phosphorylation catalyzed by ATP synthase seems to be the *rate-limiting step* for the entire process of electron transport and oxidative phosphorylation.

To continue oxidative phosphorylation, ADP is continuously translocated from the cytoplasm to the mitochondrial matrix by a *carrier-mediated facilitated exchange diffusion* because the inner membrane is not freely permeable to ADP or ATP. The carrier is a dimeric integral protein of the inner membrane and is called the *ATP-ADP translocase* (MW 58 kdal). It simultaneously transports ADP from the cytoplasm to the mitochondrial matrix, and ATP from the matrix to the cytoplasm. This *antiport* normally maintains the $[ADP]/[ATP]$ ratio in the matrix about 10 times that in the cytoplasm. The plant glycoside called *atractyloside* and the mould

antibiotic *bongkrelic acid* inhibit the ATP-ADP translocase to reduce both ADP influx and ATP outflow through the inner membrane, consequently lower the ADP level inside mitochondria and inhibit oxidative phosphorylation.

Chemiosmotic theory

According to the *chemiosmotic theory* of Peter Mitchell, phosphorylation is coupled with mitochondrial electron transport by a proton gradient maintained across the inner membrane. This proton gradient results from the translocation of protons from the mitochondrial matrix to the cytoplasmic side of the membrane by the respiratory chain.

The inner membrane is impermeable to protons. But during the transport of a pair of electrons along the mitochondrial respiratory chain, 3 pairs of protons are translocated from the inner to the outer side of the membrane. This proton translocation is made possible by several factors: (i) Some of the respiratory chain components like the flavoproteins and coenzyme Q accept and transport both protons and electrons while others like the iron-sulfur proteins and cytochromes accept and carry only electrons. (ii) Membrane-bound respiratory chain complexes, viz., NADH-Q reductase, QH_2 -cytochrome c reductase and cytochrome oxidase, span the entire thickness of the inner membrane so that they can accept protons from one side of the membrane and release them on the other side. (iii) The respiratory chain complexes are asymmetrically oriented in the membrane—electron-carriers that accept both protons and electrons are located on the matrix side of the membrane while electron-carriers accepting only electrons and no proton occur on the cytoplasmic side. (iv) Because of such locations and orientations, electron-carriers accepting both protons and electrons take up both these from the matrix side of the inner membrane. But

they release the protons on the cytoplasmic side of the membrane when they transfer only the electrons to other electron-carriers accepting no proton. Thus, an inward proton gradient is created across the inner membrane.

More than one *redox loop* are constituted in the membrane, each with electron-carriers transporting electrons from the inner to the outer side of the membrane and back again to the inner side (Fig. 10.17). Electron transport through a redox loop pumps out protons to the cytoplasmic side of the membrane. This proton translocation maintains a higher H^+ concentration outside the inner membrane, a pH gradient (ΔpH) with the outside pH lower by 1.4 units than the inside pH, and also a membrane potential ($\Delta\psi$) of 0.14 volt with electronegativity on the inner side of the membrane. The total electrochemical potential (ΔP), resulting from ΔpH and $\Delta\psi$, is utilized by ATP synthase.

$$\begin{aligned}\Delta P &= \Delta\psi - \frac{RT}{F} \Delta pH \\ &= 0.14 - 0.059(-1.4) \\ &= 0.224 \text{ V}\end{aligned}$$

where R is the molar gas constant amounting to 8.314×10^7 ergs degree $^{-1}$ mol $^{-1}$, T is 298 K (25°C) and F is the calorie equivalent of the faraday amounting to 23.06 kcal mol $^{-1}$.

An earlier version of the chemiosmotic theory proposed three redox loops, each translocating two protons per pair of electrons transported (Fig. 10.17). The *first loop* is constituted by the NADH-Q reductase complex. Its FMN accepts a hydride ion (H^-) from NADH and a proton from the matrix on the inner side of the membrane to change into $FMNH_2$. The latter liberates two protons on the outer side of the membrane, but donates two electrons to an iron-sulfur protein which carries them to the inner side of the membrane. The *second loop* is constituted by the QH_2 -cytochrome c

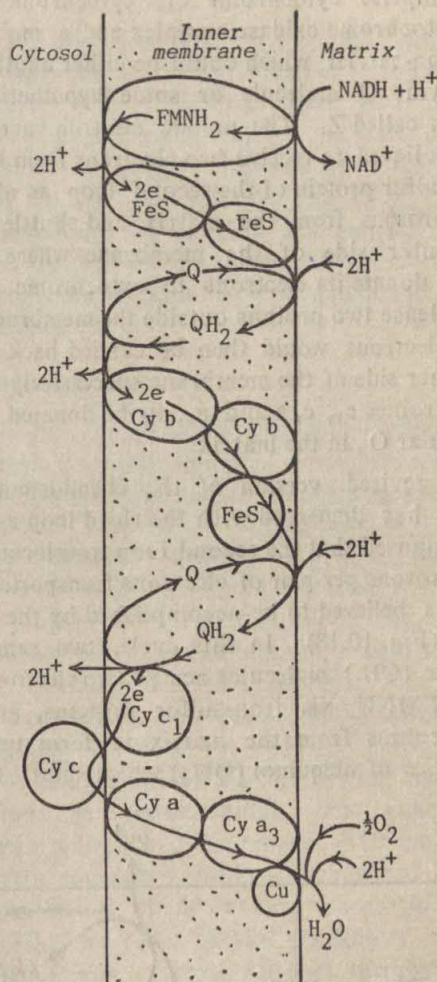


Fig. 10.17. Proposed redox loops in the electron transport chain.

reductase complex. The mobile coenzyme Q molecule receives electrons from the iron-sulfur protein of the first loop on the inner side of the membrane, accepts two protons from the matrix and gets reduced to QH_2 . The latter diffuses to the outer side of the membrane and donates its electrons to cytochrome b molecules, but releases two protons outside the membrane. Cytochrome b brings the electrons back to the inner side of the membrane and transfers them to an iron-sulfur protein. The *third loop* was believed

to comprise cytochrome c_1 , cytochrome c , the cytochrome oxidase complex and a mobile electron-carrier which could be either another coenzyme Q molecule or some hypothetical carrier called Z. The mobile electron carrier was believed to receive two electrons from the iron-sulfur protein of the second loop as also two protons from the matrix and shuttle to the outer side of the membrane where it would donate its electrons to cytochrome c_1 and release two protons outside the membrane. The electrons would then be carried back to the inner side of the membrane successively by cytochromes c_1 , c , a and a_3 , to be donated to molecular O_2 in the matrix.

A revised version of the chemiosmotic theory has dispensed with the third loop and has proposed that the second loop translocates four protons per pair of electrons transported. This is believed to be accomplished by the *Q cycle* (Fig. 10.18). In this cycle, two semiquinone (QH.) molecules accept two electrons from $FMNH_2$ via iron-sulfur proteins, and two protons from the matrix to form two molecules of ubiquinol (QH_2) which shuttle to

the outer side of the membrane. There they donate two electrons to two cytochrome b molecules and release two protons outside the membrane, changing into semiquinones (QH.) again. The latter donate two electrons to two cytochrome c_1 molecules and two protons outside the membrane, changing back to two ubiquinone (Q) molecules. The Q molecules shuttle back to the inner side of the membrane where they receive two protons from the matrix and two electrons from two reduced cytochrome b molecules to form two semiquinone (QH.) molecules again. This process is repeated to run the cycle.

Cytochrome oxidase by itself does not seem to be equipped to translocate protons across the membrane because its metal ions can accept and donate only electrons and no proton. Nevertheless, experimental evidences indicate strongly that it functions as the third site for the transmembrane proton translocation though not as a part of a redox loop. It has been proposed that cytochrome oxidase acts as a *proton pump* and actively pumps out protons from the matrix to the cytosol through

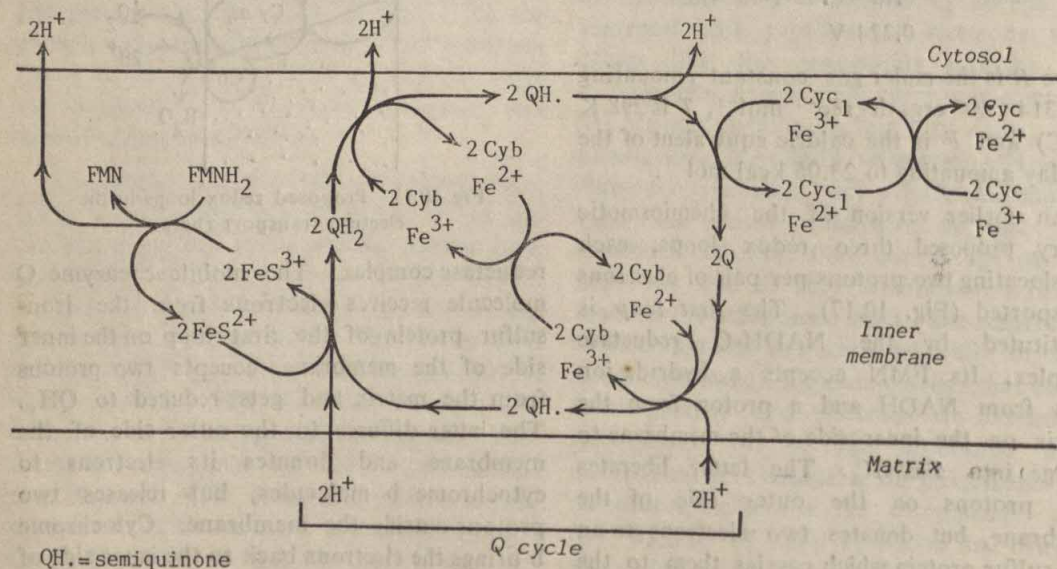


Fig. 10.18. The Q cycle.

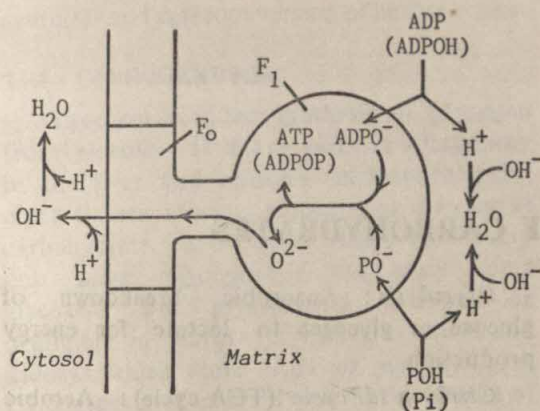


Fig. 10.19. Mitchell hypothesis about ATP synthesis by the F_1-F_0 complex.

an ion channel within its oligomeric apoprotein, utilizing the free energy released during the transport of electrons by this enzyme.

Each site for proton translocation helps to generate a high-energy bond of ATP per pair of protons translocated. The utilization of the proton gradient in ATP synthesis has been sought to be explained by a hypothesis that the proton gradient helps only indirectly to drive the ATP synthase in forming ATP. Due to the electrochemical gradient resulting from a higher proton concentration outside the inner membrane than inside it, protons may pass from outside the membrane to the F_1 unit of the inner membrane particle through a proton channel in its F_0 unit. This may induce conformational changes in the F_1 unit to enhance its action as ATP synthase in phosphorylating ADP to ATP. But according to another hypothesis proposed by Mitchell, protons and hydroxyl ions (from O^{2-}) produced during the phosphorylation of ADP flow respectively to the matrix side and the

cytoplasmic side of the inner membrane because the proton gradient keeps the concentration of protons lower than that of the hydroxyl ions on the matrix side of the membrane and higher than that of the hydroxyl ions on the cytoplasmic side (Fig. 10.19). This removal of protons and hydroxyl ions from the site of the reaction drives the phosphorylation reaction by lowering the product concentrations.

Uncouplers

Some lipid-soluble phenolic compounds such as 2,4-dinitrophenol, pentachlorophenol and dinitrocresol act as uncouplers of mitochondrial respiration and phosphorylation. They reduce or nullify the proton gradient across the inner membrane by probably carrying protons from the cytosol to the mitochondrial matrix. Consequently, they inhibit ATP formation by ATP synthase. But they do not inhibit mitochondrial respiration. They also promote the ATPase activity of the F_1 unit by enhancing the proton concentration in mitochondria. The enhanced ATPase activity and the reduced ATP synthase activity result in a rise in mitochondrial ADP concentration which in turn brings about a manifold increase in the respiratory chain activity. The energy, released thereby but not utilized in ATP synthesis, is dissipated as heat.

Other uncouplers include thyroid hormones and dicumarol, the vitamin K antagonist. In hibernators, infants and adult mammals exposed to cold, noradrenaline is secreted and it increases the lipolysis of brown fat to fatty acids which uncouple mitochondrial respiration and phosphorylation to generate heat.

11. METABOLISM OF CARBOHYDRATES

Carbohydrates are mainly utilized for energy production. They are also used in synthesizing glycolipids, glycoproteins, mucoproteins, nucleotides, nucleic acids, glycerol, porphyrins and many amino acids. Carbohydrates are obtained mainly from food, but are also produced in the body from amino acids, glycerol, propionate and lactate. Major metabolic pathways of carbohydrates are as follows (Fig. 11.1).

Glycogenesis : Synthesis of glycogen from glucose for storage.

Glycogenolysis : Breakdown of liver glycogen to glucose.

Glycolysis : Anaerobic breakdown of glucose or glycogen to lactate for energy production.

Citric acid cycle (TCA cycle) : Aerobic metabolism of glycolytic products for energy production.

Pentose phosphate pathway : Pathway for the synthesis and catabolism of pentoses and the generation of NADPH.

Gluconeogenesis : Synthesis of carbohydrates from noncarbohydrates such as amino acids, lactate and glycerol.

Uronic acid pathway : Synthesis of hexuronic acids from glucose.

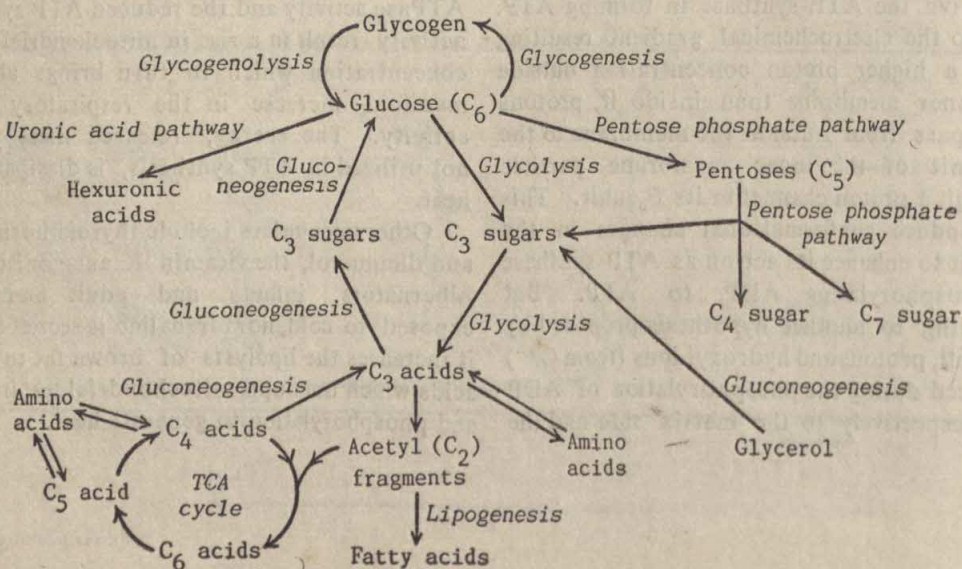


Fig. 11.1. Metabolic pathways of carbohydrates.

Amino sugar pathway: Pathway for the synthesis and interconversion of hexosamines.

11.1 GLYCOGENESIS

Glycogenesis is the synthesis of glycogen from glucose. It takes place in a large way in the liver and muscles which are the principal tissues storing glycogen as the reserve carbohydrate. Most other tissues also carry out some glycogenesis and store some glycogen. But glycogenesis is absent or negligible in mature erythrocytes, brain and kidneys which store little or no glycogen. Glycogenesis is carried out in the cytosol of the cell.

Reaction sequence

(a) Glucose is phosphorylated to glucose 6-phosphate with the help of ATP, Mg^{2+} and either hexokinase or glucokinase (Fig. 11.2). The enzyme transfers the terminal γ -phosphate group of the Mg^{2+} -ATP complex to the C⁶-hydroxyl group of glucose, producing Mg^{2+} -ADP and glucose 6-phosphate. The

reaction is practically irreversible in the physiological system. ($\Delta G^\circ \approx -4 \text{ kcal mol}^{-1}$).

Hexokinases are relatively less specific for the substrate and can phosphorylate α - and β -D-glucose, other D-hexoses, D-glucosamine and 2-deoxy-D-glucose. Hexokinases possess low K_m ($50 \mu\text{M litre}^{-1}$) and high affinities for glucose, much higher K_m for other hexoses and a high V_{max} . Hexokinase isozymes III, I and II have glucose-affinities in a decreasing order and undergo feed-back allosteric inhibition by glucose 6-phosphate, the product of their action.

Glucokinase is an inducible enzyme acting specifically on glucose and is not allosterically inhibited by glucose 6-phosphate. It has a far higher K_m (10 mM litre^{-1}) for glucose than hexokinases and consequently catalyzes glycogenesis and glucose uptake only at blood sugar levels of 100 mg dl^{-1} or higher, as happen after a meal. Glucokinase predominates over hexokinases in the liver; hexokinases predominate in extrahepatic tissues and their low K_m for glucose helps

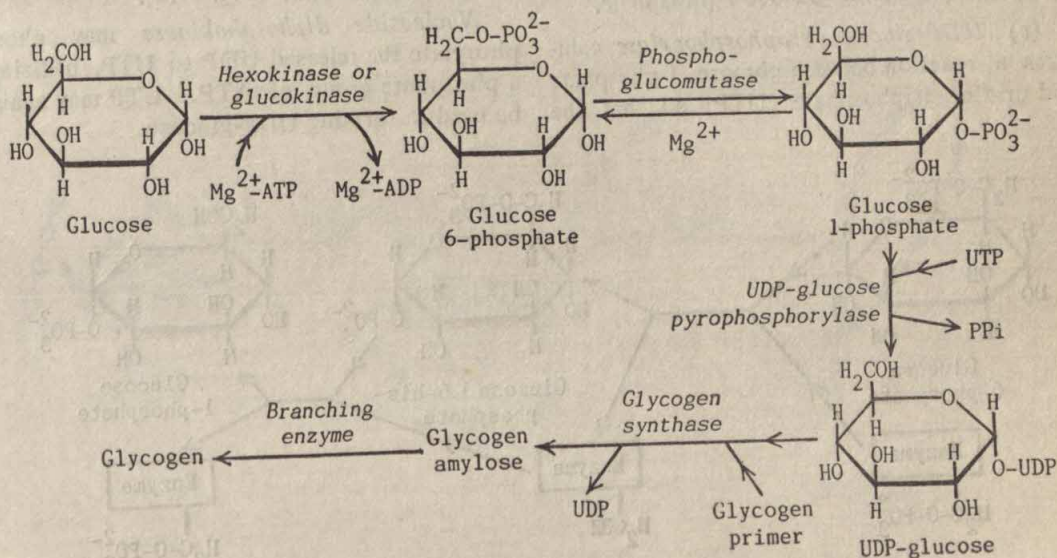


Fig. 11.2. Pathway of glycogenesis.

muscles to take up glucose even at low blood sugar levels.

The molecular conformation of hexokinase changes on binding with glucose—an example of the *induced fit* model (see page 94). This closes the cleft between the two lobes of the enzyme molecule, causing the nonpolar amino acid sidechains to surround the glucose molecule except its C⁶-hydroxymethyl group. This facilitates the phosphorylation of that group with ATP and prevents the binding of H₂O with the substrate-binding site and the hydrolysis of ATP.

(b) *Phosphoglucumutase* next isomerizes glucose 6-phosphate to glucose 1-phosphate in presence of Mg²⁺ and catalytic amounts of glucose 1,6-bisphosphate (glucose 1,6-diphosphate). The enzyme first transfers a phosphate group from a phosphoserine residue of its active site to the C¹ of glucose 6-phosphate to change the latter to a glucose 1,6 bisphosphate intermediate (Fig. 11.3). Next, it transfers a phosphate group from the C⁶ of the intermediate to the serine residue at the active site, changing the serine back to phosphoserine and producing glucose 1-phosphate.

(c) *UDP-glucose pyrophosphorylase* catalyzes a reaction between glucose 1-phosphate and uridine triphosphate (UTP) so that the

two terminal phosphate groups of UTP are released as inorganic pyrophosphate (PPi) and glucose 1-phosphate occupies their place to form uridine diphosphoglucose (UDP-glucose) (Fig. 11.2). A *pyrophosphatase* immediately hydrolyzes PPi; this drives the reaction towards the synthesis of UDP-glucose.

UDP-glucose is a highly reactive complex of glucose and has a phosphoryl-glycoside bond of ΔG° about -7 kcal mol^{-1} . It serves as a donor of glucose for incorporation of the latter into polysaccharides, oligosaccharides and gangliosides.

(d) *Glycogen synthase* transfers glucose from UDP-glucose to the nonreducing end of a pre-existing oligosaccharide (*glycogen primer*) having more than four glucose residues. The C¹ of the added glucose binds to the C⁴ of the pre-existing terminal glucose residue of a primer chain by an α -1,4 glycosidic bond. Repeated glycogen synthase action forms unbranched polyglucose chains (glycogen amylose or α -1,4-glucan), adding one glucose molecule and releasing one UDP molecule at each step (Fig. 11.2).

Nucleoside diphosphokinase may phosphorylate the released UDP to UTP, utilizing a phosphate group of ATP. UTP may again be used in forming UDP-glucose.

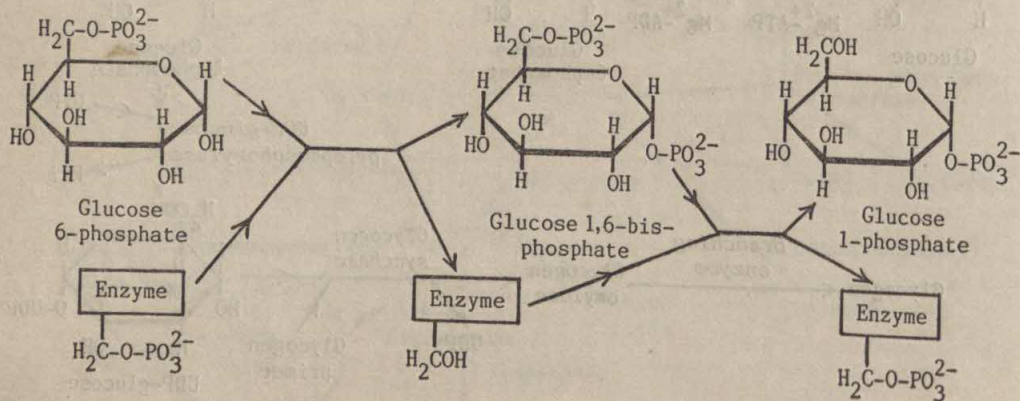


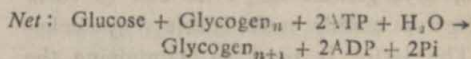
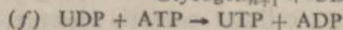
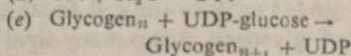
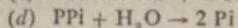
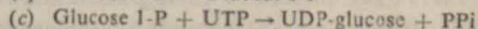
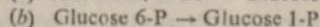
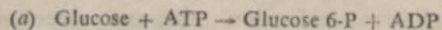
Fig. 11.3. Mode of action of phosphoglucumutase.

(e) After repeated glycogen synthase action has extended the chain length by 11 or more glucose residues, *branching enzyme* (amyl-1,4 $\rightarrow$ 1,6-transglucosidase) splits an α -1,4 glycosidic bond of the chain and transfers the released oligosaccharide of 6 or more glucose units to such a site on a primer chain as is separated by 8-12 glucose residues from a pre-existing branching point (Fig. 11.4). The transferred oligosaccharide is linked by a 1,6-glycosidic bond to the C⁶ of a glucose residue of the chain at the new site, producing a new 1,6 branching. This action is repeated on other chains to change the unbranched glycogen amylose molecule into the branched molecule of glycogen.

Energy expenditure

During glycogenesis, one high-energy phosphate bond ($\sim$ P) of ATP is spent in phosphorylating glucose to glucose 6-phosphate. Subsequently, UTP loses two $\sim$ P bonds as PPi in forming UDP-glucose, but regains one $\sim$ P bond when it is released as UDP during glycogen synthase action. Finally, UDP regains another $\sim$ P bond also from ATP

which, however, loses a $\sim$ P bond during this reaction.



Hence, two high-energy phosphate bonds of ATP are spent in incorporating each glucose molecule into glycogen in glycogenesis.

Significance

Glycogenesis forms the storage polysaccharide glycogen from such glucose as is in excess of the body's immediate need. It thus helps to bring down the blood sugar level from its peak after a carbohydrate meal. Liver and muscle cells store glycogen in electron-dense cytoplasmic granules of diameter about 10-40 nm. The polymerization of

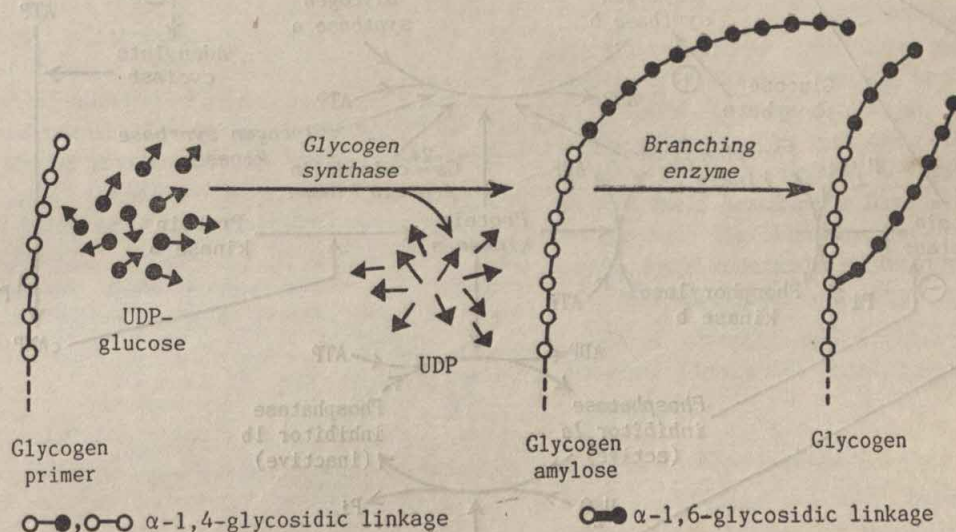


Fig. 11.4. Actions of glycogen synthase and the branching enzyme.

glucose to glycogen reduces the bulk of the stored nutrient by eliminating water molecules from glucose molecules. Because glycogen has far larger molecules than glucose and is stored mainly as particulate cytoplasmic granules, glycogenesis enables the cell to store large amounts of glycogen without any significant rise in intracellular osmotic pressure. Besides, glycogenesis helps the cell to form and store a readily mobilizable polysaccharide. The latter is used in maintaining the blood sugar level in spite of the day-to-day variations in the food intake and during the first 24 hours of fasting; it also contributes to energy production during intense activity.

Extensive branchings of the glycogen molecule help the actions of glycogen synthase and glycogen phosphorylase because both act only at the nonreducing ends of the branches.

Glycogen can be speedily metabolized according to the body needs, because both

glycogenic and glycogenolytic enzymes occur in glycogen granules.

Regulation

Glycogenesis is largely regulated by the reversible covalent modifications of glycogen synthase through its phosphorylation and dephosphorylation.

Glycogen synthase catalyzes the *rate-limiting step* of glycogenesis. It exists in two forms in muscles (Fig. 11.5): (i) *Glycogen synthase b* (synthase D): This is the less active and phosphorylated form; it carries phosphoserine residues and needs for its action a lowering of its K_m for UDP-glucose by the allosteric action of glucose 6-phosphate. (ii) *Glycogen synthase a* (synthase I): This is the highly active and dephosphorylated form which carries no phosphate on its serine residues and needs no glucose 6-phosphate for its activity. Active synthase *a* is changed to

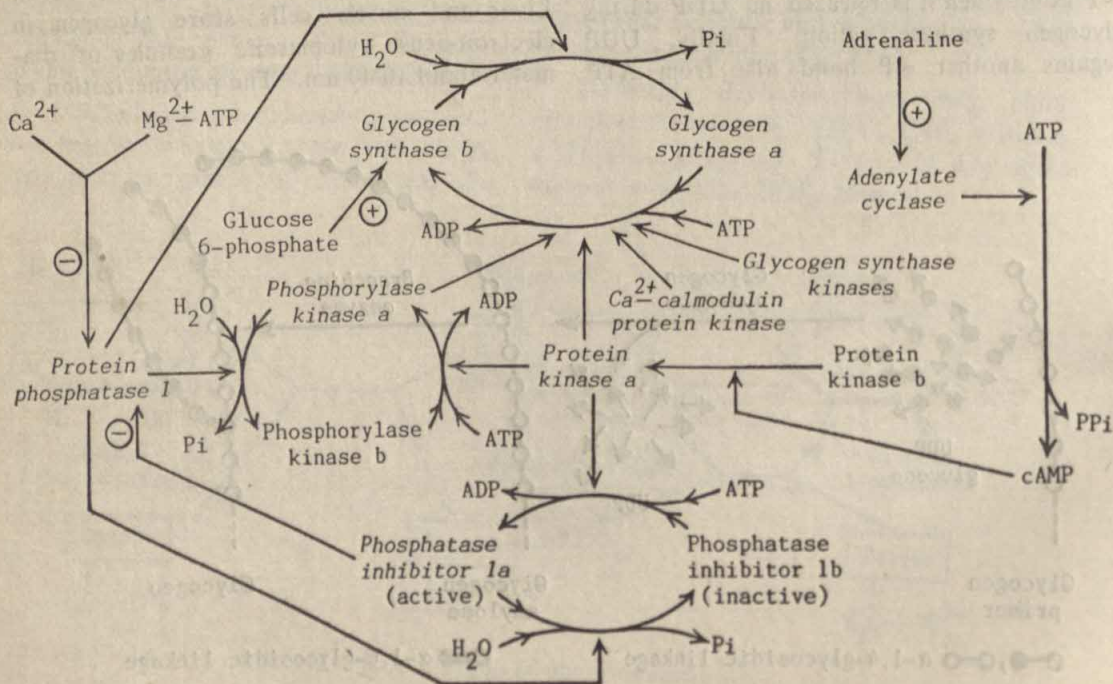


Fig. 11.5. Regulation of glycogenesis.

inactive synthase *b* by the phosphorylation of serine residues at its seven sites (*multisite phosphorylation*) with the help of ATP and enzymes such as a cAMP-dependent *protein kinase*, a *phosphorylase kinase*, and three *glycogen synthase kinases*. As more sites of glycogen synthase get phosphorylated, its K_m for UDP-glucose rises progressively and its activity declines progressively, requiring increasingly higher levels of glucose 6-phosphate for its allosteric activation. On the other hand, *protein phosphatase-1* hydrolyzes the phosphoester bonds in glycogen synthase *b* to give active glycogen synthase *a* and P_i . Protein phosphatase-1 is, in turn, inhibited by a combination of Mg^{2+} -ATP and Ca^{2+} or by the *phosphatase inhibitor protein-1* which is activated by phosphorylation with ATP and protein kinase *a* (Fig. 11.5).

Adrenaline binds with β -adrenergic receptors on the muscle cell membrane and activates *adenylate cyclase* there. This initiates a *cascade system*, leading to the inactivation of very many molecules of glycogen synthase and a decline in glycogenesis (Fig. 11.5). Adenylate cyclase increases intracellular cyclic AMP (cAMP) by changing ATP to cAMP and P_{Pi} . cAMP binds with allosteric subunits (A) of inactive protein kinase *b* (A_2C_2), forming cAMP-allosteric subunit complexes ($cAMP_2A$) and releasing the catalytic subunits (C) of the enzyme as active protein kinase *a* molecules: $A_2C_2 + 4cAMP \rightleftharpoons 2C + 2cAMP_2A$. The protein kinase *a* phosphorylates three sites of glycogen synthase *a* and also phosphorylates inactive phosphorylase kinase *b* to active phosphorylase kinase *a* which in turn phosphorylates another site of glycogen synthase *a*. These phosphorylations change glycogen synthase *a* progressively to inactive synthase *b*. Simultaneously, protein kinase *a* activates phosphatase inhibitor-1 which in turn inhibits protein phosphatase-1 to prevent the dephosphorylation of glycogen synthase *b*. All these reduce the rate of glycogenesis.

Protein kinase *a* and phosphorylase kinase *a* actions serve to phosphorylate and thereby activate glycogen phosphorylase for glycogen breakdown; on the contrary, protein phosphatase-1 dephosphorylates glycogen phosphorylase also, but thereby inactivates it. These reciprocal effects of these enzymes on glycogen synthase and phosphorylase ensure that an increased rate of glycogenesis is accompanied by a decreased rate of glycogen breakdown and vice versa.

Glycogenesis and glycogen breakdown are reciprocally regulated in the liver also by the protein kinase, phosphorylase kinase and protein phosphatase-1.

In both muscles and liver, a high glycogen level causes a feed-back inhibition of protein phosphatase-1 and consequently keeps glycogen synthase in the inactive phosphorylated form, thereby reducing glycogenesis. But liver requires a far higher glycogen level than muscles for such feed-back inhibition. So, glycogen synthase continues glycogenesis in the liver, enabling it to remove glucose from blood, long after muscles have ceased doing so due to a rise in the muscle glycogen level.

Because of its high K_m for glucose, liver glucokinase cannot function at low levels of blood sugar, thereby reducing hepatic glycogenesis and sparing the sugar for other tissues. But muscles and other tissues still continue glucose uptake and glycogenesis because their hexokinases have far lower K_m for glucose. Hexokinases, but not glucokinase, are allosterically inhibited by the feed-back effect of glucose 6-phosphate.

Insulin induces the synthesis of glucokinase and glycogen synthase, thereby enhancing glycogenesis.

11.2 GLYCOGENOLYSIS

Glycogenolysis is the breakdown of glycogen to glucose. It takes place mainly in the liver.

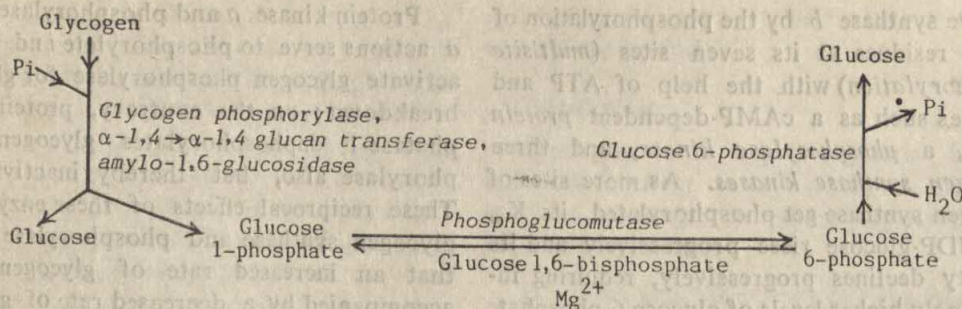


Fig. 11.6. Pathway of glycogenolysis.

Reaction sequence

The last step of this process takes place on the smooth endoplasmic reticulum membrane of liver cells. Steps preceding it take place in the cytosol of liver cells.

(a) *Glycogen phosphorylase* catalyzes the phosphorolysis of the α -1,4 glycosidic bonds in the stored glycogen with the help of inorganic phosphate. This breaks the α -1,4 bonds between successive glucose residues starting from the nonreducing end of an outer chain of glycogen molecule and releases one glucose 1-phosphate molecule at each step (Fig. 11.6). The enzyme, however, cannot cleave the α -1,6 glycosidic bonds at the

branching points of glycogen chains. Its repeated action on successive α -1,4 bonds in a branch of the glycogen molecule progressively shortens that branch (Fig. 11.7). *Glycogen phosphorylase* stops acting on a branch when only four glucose residues are left in it.

(b) α -1,4 $\rightarrow$ α -1,4 *glucan transferase* then cleaves the last 1,4 glycosidic bond before the α -1,6 glycosidic bond at the branching point of the shortened branch, transfers a trisaccharide unit from that branch to another branch, and joins the trisaccharide to the latter by a new α -1,4 glycosidic bond (Fig. 11.7). This leaves only the last glucose

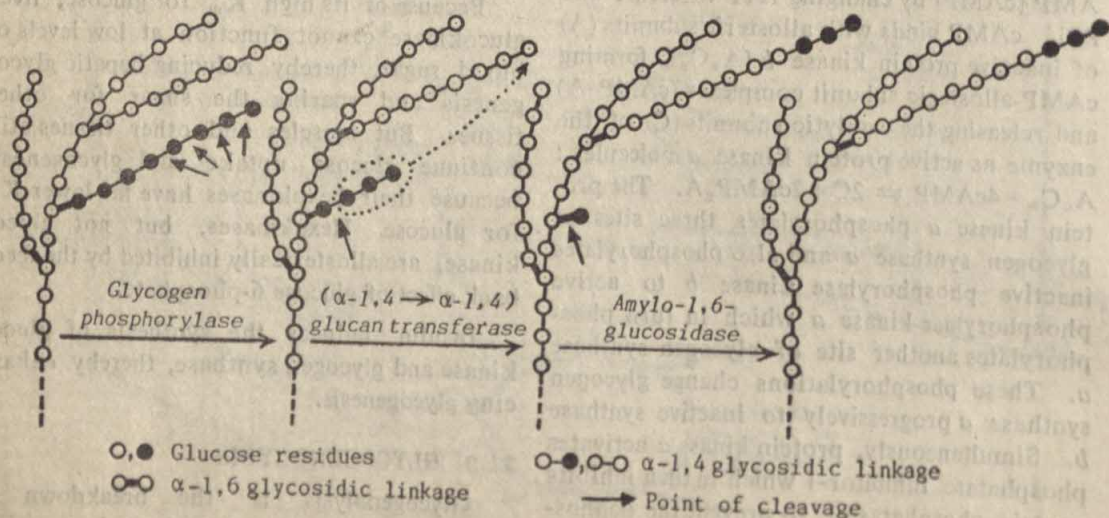


Fig. 11.7. Actions of glycogen phosphorylase, glucan transferase and amylo-1,6-glucosidase in glycogenolysis.

residue of the shortened branch still attached to the branching point by the α -1,6 glycosidic bond.

(c) *Amylo-1,6-glucosidase* (debranching enzyme) hydrolyzes the α -1,6 glycosidic bond connecting the last glucose residue of the shortened branch to its branching point, and releases that residue as free glucose.

Reactions (a), (b) and (c) are repeated on other branches of the glycogen molecule. About 90% of the glucose residues of glycogen are released as glucose 1-phosphate by glycogen phosphorylase while the remaining 10% are released as glucose by amylo-1,6-glucosidase.

(d) *Phosphoglucomutase* next isomerizes glucose 1-phosphate to glucose 6-phosphate. The enzyme transfers a phosphate group from

its phosphoserine residue to the C⁶-hydroxymethyl group of glucose 1-phosphate, changing the latter to a glucose 1,6-bisphosphate intermediate (Fig. 11.8). A phosphate group is next transferred from the C¹ of the intermediate to the serine residue of the enzyme, reconvert that residue to phosphoserine and producing glucose 6-phosphate. The enzyme requires Mg^{2+} and catalytic amounts of glucose-1,6-bisphosphate for its action.

(e) Glucose 6-phosphate cannot pass into the blood through the plasma membrane. Liver cells possess *glucose 6-phosphatase* in their microsomal membrane; this enzyme hydrolyzes glucose 6-phosphate into Pi and glucose, the latter passing out from the cell into the blood.

Due to the absence of glucose 6-phosphatase, muscles and brain can neither carry out glycogenolysis nor add glucose to the blood. Very little glycogenolysis takes place in kidneys because the renal cells, though containing glucose 6-phosphatase, store very little glycogen; instead, glucose 6-phosphatase helps in gluconeogenesis in kidneys.

Significance

Hepatic glycogenolysis adds glucose to the blood for maintaining the blood sugar level inspite of the day-to-day variations in the food intake. It is also important for maintaining the blood sugar level during the first 24 hours of fasting. But if fasting continues even after that, hepatic glycogen store is practically spent up and glycogenolysis loses its importance in maintaining the blood sugar.

Regulation

Glycogen phosphorylase is the enzyme for the *rate-limiting step* of glycogenolysis. Glycogenolysis is largely regulated by the reversible covalent modification of glycogen phosphorylase through its phosphorylation and dephosphorylation.

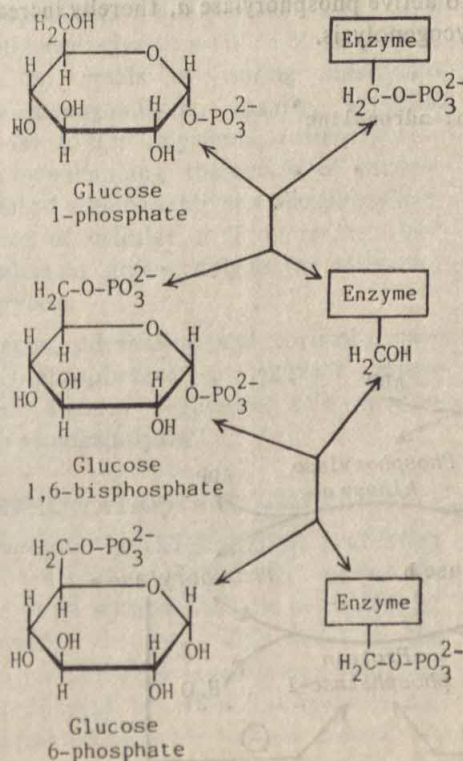


Fig. 11.8. Action of phosphoglucomutase in glycogenolysis.

Adrenaline as well as glucagon rapidly increases glycogenolysis manifold by initiating a *cascade system* in the liver (Fig. 11.9). *Adenylate cyclase* is activated in the hepatocyte membrane by the binding of adrenaline or glucagon with β -adrenergic and glucagon receptors respectively on the membrane. *Adenylate cyclase* changes ATP to cAMP in the cell. cAMP allosterically changes inactive protein kinase *b* to active *protein kinase a* (see page 313). The latter phosphorylates inactive phosphorylase kinase *b* to active *phosphorylase kinase a* with the help of ATP. *Phosphorylase kinase a* then uses ATP to phosphorylate a specific serine residue of inactive phosphorylase *b*, thereby changing the latter to active *glycogen phosphorylase a*.

On the other hand, *protein phosphatase-1* hydrolyzes the phosphoester bonds in both phosphorylase *a* and phosphorylase kinase *b* to convert them to Pi and the respective

inactive enzymes. But *protein phosphatase-1* is inhibited by phosphorylase *a* and *phosphatase inhibitor-1*, the latter being phosphorylated and activated by *protein kinase a*.

It follows, therefore, that glucagon secreted during starvation and adrenaline secreted during stress, can enhance glycogenolysis through the activation of glycogen phosphorylase and the simultaneous inhibition of *protein phosphatase-1*.

On the contrary, on binding with α_1 adrenergic receptors on the hepatocyte membrane, adrenaline increases the translocation of Ca^{2+} from mitochondria to the cytosol, without affecting the cellular cAMP level (Fig. 11.10). Binding of Ca^{2+} with the cytoplasmic protein *calmodulin* activates a *calmodulin-sensitive phosphorylase kinase* which in turn phosphorylates phosphorylase *b* to active phosphorylase *a*, thereby increasing glycogenolysis.

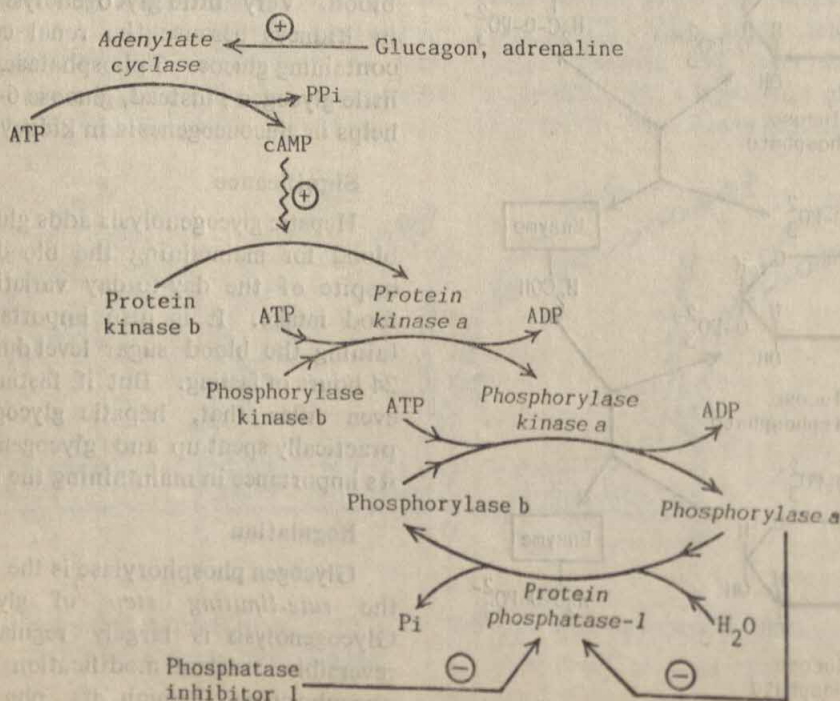


Fig. 11.9. The cAMP-dependent cascade system regulating glycogen phosphorylase.

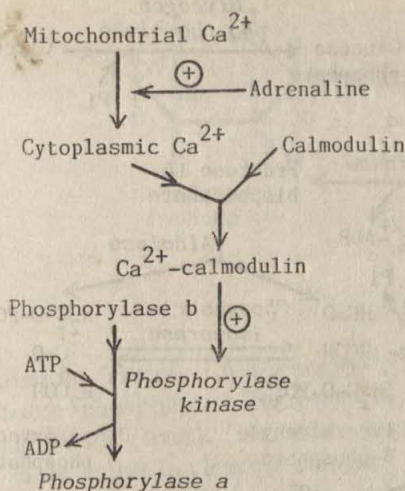


Fig. 11.10. Ca^{2+} -calmodulin action in regulating glycogen phosphorylase.

Glucose inhibits phosphorylase allosterically. So, a rise in blood glucose after a meal inhibits glycogenolysis; a fall in blood glucose between the meals or during starvation enhances glycogenolysis. Insulin, secreted in response to hyperglycemia, reduces glycogenolysis by enhancing the action of protein phosphatase-1 which inactivates phosphorylase. A depletion of cellular ATP increases AMP which enhances glycogenolysis by activating phosphorylase.

Glucagon, adrenaline and cortisol induce glucose 6-phosphatase to enhance glycogenolysis. Insulin represses this enzyme to reduce glycogenolysis.

11.3 FERMENTATION AND GLYCOLYSIS

Fermentation is the process of generating metabolic energy as high-energy phosphate bonds of ATP without the use of molecular O_2 (anaerobic process). This involves the oxidation of organic substrates, but the electrons donated by them during the oxidation are accepted by other organic substances instead of molecular O_2 . The substrates for fermentation are mainly carbohydrates such as

glucose, lactose, maltose, sucrose, glycogen and cellulose, according to the species. Reactions of the process are remarkably similar in a vast number of species. But fermentation cannot completely oxidize the substrate; there is further scope of aerobic oxidation of its endproducts. Endproducts of fermentation vary from species to species, e.g., lactic acid in animal tissues and *Lactobacilli*, acetic acid in acetic acid bacteria, and ethanol and small amounts of glycerol in yeast.

Glycolysis

Glucose and glycogen are anaerobically catabolized (fermented) in animal tissues to pyruvate and lactate through the *Embden-Meyerhoff-Parnas pathway* of glycolysis. It is the principal energy-generating pathway in erythrocytes, white striated muscle fibres, brain, skin, renal medulla, retina, lens, cornea, and gastrointestinal tract (Fig. 11.11). The glycolytic enzymes occur in the cytosol.

Reaction sequence :

(a) Muscles, brain, kidneys and erythrocytes collect glucose from the blood and glycolyze that glucose directly. In such cases, hexokinase phosphorylates glucose to glucose 6-phosphate with the help of ATP (see page 309). Hexokinases have low K_m values like $50 \mu\text{M litre}^{-1}$ for glucose; so, muscles can take up glucose from the blood and glycolyze it even when the blood sugar level is relatively low. This hexokinase-mediated phosphorylation is practically irreversible in the physiological system ($\Delta G^\circ' = -4 \text{ kcal mol}^{-1}$). It involves the expenditure of one high-energy phosphate bond of ATP per glucose molecule changed.

Muscles may also glycolyze stored glycogen. In that case, glycogen phosphorylase phosphorylates glycogen into glucose 1-phosphate molecules with the help of P_i in the same way as in hepatic glycogenolysis (see page 314). However, muscle glycogen

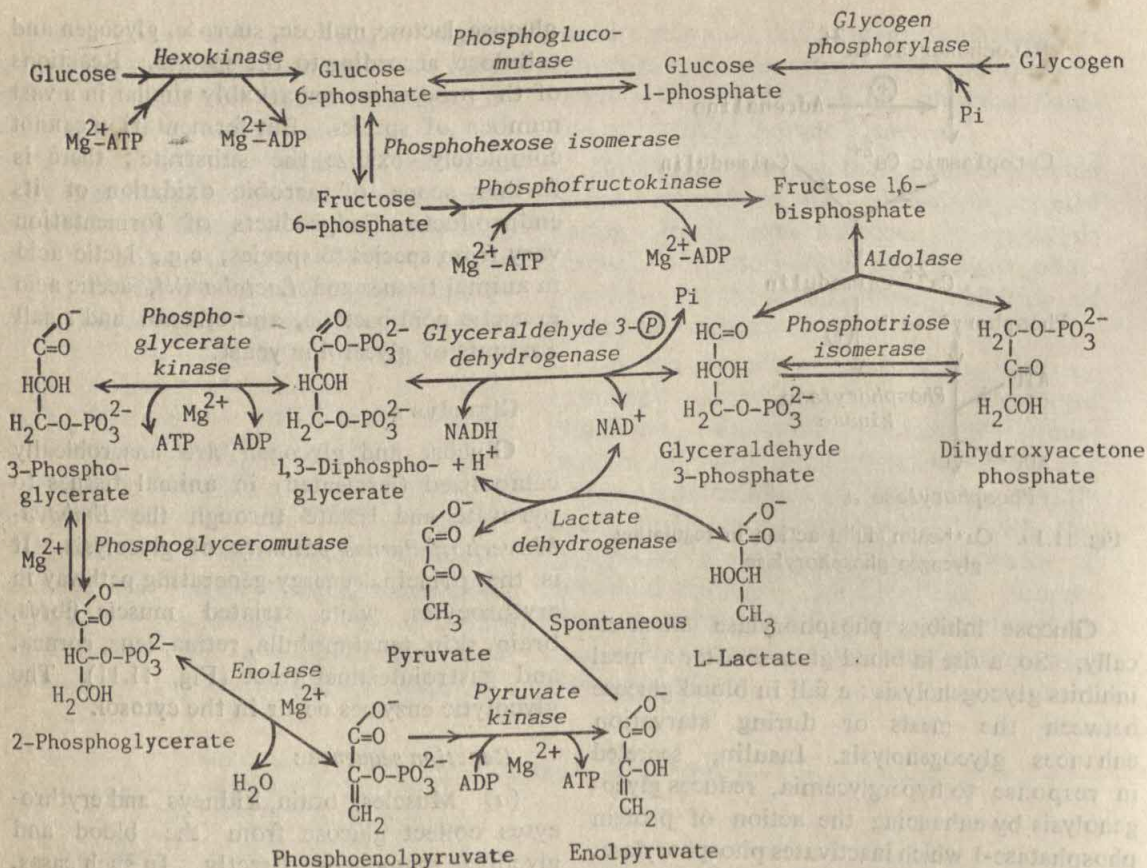


Fig. 11.11. Pathway of glycolysis.

phosphorylase is different from the liver enzyme and bears a pyridoxal phosphate molecule on each of its 92-kdal peptide chains. Instead of the aldehyde group of pyridoxal phosphate, its phosphoryl group seems to participate directly in the catalysis. *Phosphoglucomutase* next isomerizes glucose 1-phosphate to glucose 6-phosphate through a glucose 1,6-bisphosphate intermediate.

(b) *Phosphohexose isomerase* isomerizes glucose 6-phosphate to fructose 6-phosphate, probably through a *cis*-1,2-enediol phosphate intermediate (Fig. 11.12). It is thus an aldose-ketose isomerase changing the aldose glucopyranose to the ketose fructofuranose.

(c) *Phosphofructokinase* (phosphofructokinase 1) phosphorylates fructose 6-phosphate to fructose 1,6-diphosphate (fructose 1,6-diphosphate) with the help of ATP and Mg^{2+} . Its action is practically irreversible ($\Delta G^{\circ} = -4.5 \text{ kcal mol}^{-1}$). This tetrameric enzyme belongs to the K series of allosteric enzymes. ATP, its allosteric inhibitor, increases its K_m for fructose 6-phosphate to reduce its activity.

(d) *Aldolase* catalyzes an aldol cleavage of fructose 1,6-bisphosphate into two triose phosphates, viz., dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. Aldolase A (muscle) and aldolase C (brain) possess higher

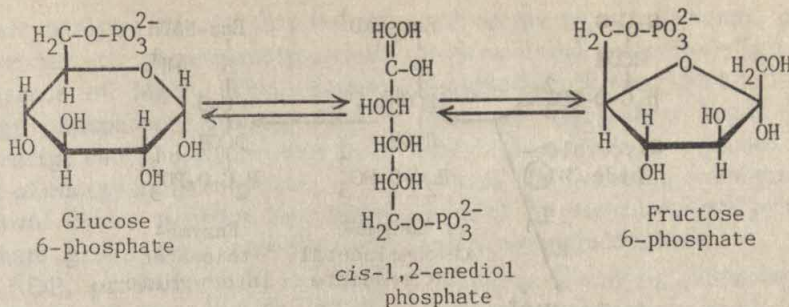


Fig. 11.12. Mode of action of phosphohexose isomerase.

affinities for fructose 1,6-bisphosphate than for the triose phosphates while reverse is the case for aldolase B (liver). So, aldolases A and C are more active in cleaving fructose 1,6-bisphosphate for glycolysis while aldolase B catalyzes mainly the reverse reaction for gluconeogenesis.

(e) *Phosphotriose isomerase*, an aldose-ketose isomerase, isomerizes dihydroxyacetone phosphate to glyceraldehyde 3-phosphate (Fig. 11.13), probably through an enediol intermediate formed with the help of the $\gamma\text{-COO}^-$ group of a glutamate residue at the active site of the enzyme. The equilibrium mixture resulting from this reversible reaction, contains 96% dihydroxyacetone phosphate and only 4% glyceraldehyde 3-phosphate. But rapid glycolytic removal of glyceraldehyde 3-phosphate moves the reaction very fast towards glyceraldehyde 3-phosphate. So this reaction, coupled with the preceding reaction (d), practically breaks each fructose

1,6-bisphosphate molecule to two molecules of glyceraldehyde 3-phosphate.

(f) *Glyceraldehyde 3-phosphate dehydrogenase* (triose phosphate dehydrogenase) catalyzes the oxidation and phosphorylation of each glyceraldehyde 3-phosphate molecule to 1,3-diphosphoglycerate (1,3-bisphosphoglycerate). NAD^+ is used as the electron-acceptor for the oxidation and is thereby reduced to NADH. This is the *only oxidative reaction* in glycolysis. Glyceraldehyde 3-phosphate dehydrogenase is a tetrameric enzyme with an SH group at its active site and a tightly bound NAD^+ molecule. The C^1 -aldehyde group of glyceraldehyde 3-phosphate first binds covalently with the SH group to form a thiohemiacetal (Fig. 11.14). A hydride ion (H^-) is next transferred from the thiohemiacetal to NAD^+ , reducing the latter to NADH and oxidizing the thiohemiacetal to a high-energy thioester intermediate. As the enzyme binds with NADH far more loosely

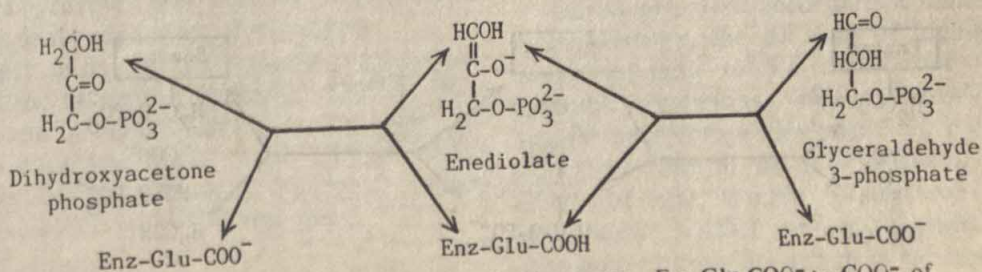


Fig. 11.13. Mode of action of phosphotriose isomerase. Enz-Glu-COO^- ; $\gamma\text{-COO}^-$ of the active site glutamate of enzyme.

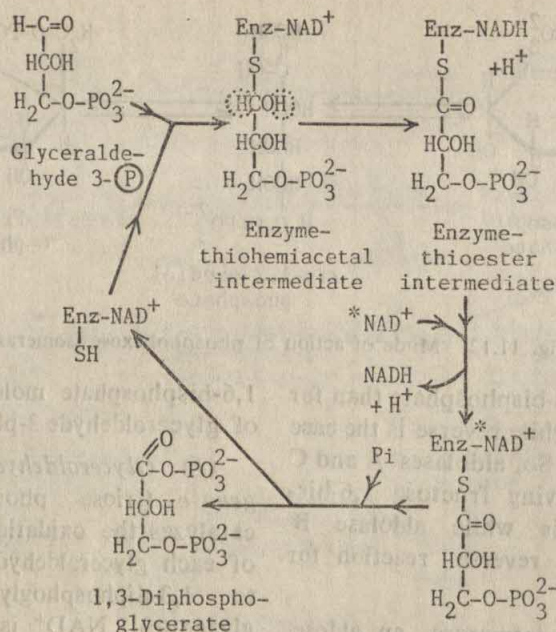


Fig. 11.14. Mode of action of glyceraldehyde 3-phosphate dehydrogenase.

than with NAD^+ , NADH leaves the enzyme-thioester intermediate and a fresh NAD^+ molecule binds in its place with the latter. The enzyme-thioester intermediate is finally phosphorylated with P_i , releasing 1,3-diphosphoglycerate and the free enzyme bearing the SH group at its active site. The energy, generated by the oxidation of the C^1 -aldehyde group of glyceraldehyde 3-phosphate, is thus used in forming a high-energy C^1 -acylphosphate group in 1,3-diphosphoglycerate.

(g) *Phosphoglycerate kinase* transfers the high-energy C^1 -phosphate group from

1,3-diphosphoglycerate to ADP , producing ATP and 3-phosphoglycerate. Thus, two high-energy phosphate bonds are produced in this step from two molecules of 1,3-diphosphoglycerate formed from each glucose molecule.

(h) *Phosphoglyceromutase* next isomerizes 3-phosphoglycerate to 2-phosphoglycerate, probably through an intermediate step of 2,3-diphosphoglycerate, utilizing a phosphate carried by a serine residue of the enzyme (Fig. 11.15). The enzyme requires Mg^{2+} and a trace of 2,3-diphosphoglycerate for its activity.

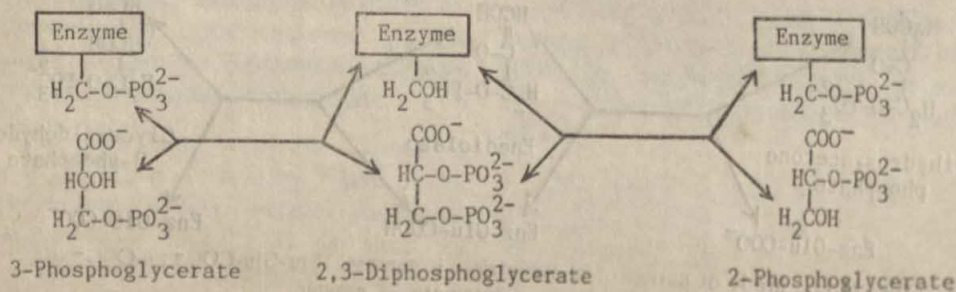


Fig. 11.15. Mode of action of phosphoglyceromutase.

(i) *Enolase* catalyzes the dehydration of 2-phosphoglycerate to phosphoenolpyruvate (PEP) in presence of Mg^{2+} . This changes the low-energy phosphoester group on its C^2 to the high-energy enol phosphate group by a redistribution of energy in the substrate.

(j) *Pyruvate kinase* transfers the high-energy phosphate group from phosphoenolpyruvate to ADP, producing ATP and enolpyruvate in presence of Mg^{2+} and K^+ . Thus, two high-energy phosphate bonds are produced in this step from two molecules of PEP formed from each glucose molecule. Enolpyruvate changes spontaneously to the keto form of pyruvate.

(k) To maintain the availability of NAD^+ for glycolysis, cytoplasmic NADH produced during the action of glyceraldehyde 3-phosphate dehydrogenase must be speedily reoxidized. In strictly anaerobic tissues such as mature erythrocytes, cutaneous epidermal cells, retina, lens, cornea, renal medulla and intensely working white muscle fibres, *lactate dehydrogenase* (LDH) oxidizes NADH in the cytoplasm by transferring its reducing equivalents (H^+ and e) to pyruvate which is thereby reduced to lactate. The latter is the final glycolytic endproduct in such cases. But aerobically respiring tissues such as red muscle fibres, cardiac muscle fibres and renal cortex reoxidize the cytoplasmic NADH by transferring its reducing equivalents to mitochondrial NAD^+ through a mitochondrial electron shuttle (see pages 300-301); this spares pyruvate from reduction and leaves it as the final endproduct of glycolysis.

Each of five LDH isoenzymes is a tetramer of H and M peptide subunits. LDH- I_4 (H_4) is present in highly aerobic cardiac and red striated muscle fibres while LDH- I_4 (M_4) occurs in predominantly glycolytic tissues such as white striated muscle fibres.

Energetics :

Glycolysis of one molecule of glucose requires the initial expenditure of two

high-energy phosphate bonds, one each at the steps catalyzed respectively by hexokinase and phosphofructokinase (PFK). The first of these two high-energy bonds is not spent when a C_6 unit from glycogen is glycolyzed because the hexokinase-catalyzed step is then replaced by steps catalyzed by phosphorylase and phosphoglucomutase.

During glycolysis producing lactate, four high-energy phosphate bonds are generated per hexose unit glycolyzed, viz., two at the phosphoglycerate kinase-catalyzed step and two more at the pyruvate kinase-catalyzed step. Deducting the number of high-energy phosphate bonds spent initially from the number of such bonds generated in glycolysis, there is a net gain of either two or three high-energy bonds from glycolysis according as a glucose molecule or a C_6 unit of glycogen has been glycolyzed (Table 11.1).

Formation of high-energy phosphate bonds by the actions of phosphoglycerate kinase and pyruvate kinase requires the formation of a high-energy metabolic intermediate (e.g., 1,3-diphosphoglycerate and phosphoenolpyruvate), coupling of its exergonic cleavage with the endergonic phosphorylation of ADP, and a direct utilization of the bond energy of the metabolic intermediate in synthesizing the high-energy bond of ATP. This process, known as the *substrate-level phosphorylation*, requires the participation of neither the mitochondrial electron transport chain nor molecular O_2 .

During glycolysis under aerobic conditions, on the contrary, the net gain of high-energy bonds amounts to 3-4 times that under anaerobic conditions. This is because *oxidative phosphorylation* (see page 302) produces additional four or six ATP bonds in consequence of the transfer of electrons from cytoplasmic NADH to mitochondria by mitochondrial shuttles and then to molecular O_2 by the mitochondrial electron transport chain (Table 11.1).

TABLE 11.1. *Production of high-energy phosphate bonds ($\sim P$) during the glycolysis of one mol of glucose.*

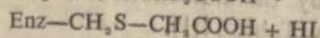
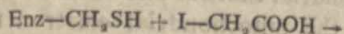
Reaction	Enzyme	$\sim P$ formed (mol)	
		O_2 absent	O_2 present
1,3-Diphosphoglycerate $\rightarrow$ 3-phosphoglycerate	Phosphoglycerate kinase	2	2
Phosphoenolpyruvate $\rightarrow$ enolpyruvate	Pyruvate kinase	2	2
$NADH \rightarrow NAD^+$	Mitochondrial enzymes and malate shuttle	0	6
	or mitochondrial enzymes and glycerophosphate shuttle	0	4
	Total	4	10 or 8*
Less mol of $\sim P$ spent for hexokinase and PFK actions		2	2
	Net gain per mol of glucose	2	8 or 6*

* according to whether the malate shuttle or the glycerophosphate shuttle has been used in transferring electrons into mitochondria from the cytoplasmic NADH.

Inhibitors:

A number of inhibitors have been used with yeast preparations and animal tissue preparations carrying out fermentations, to elucidate the steps and intermediates of the glycolytic pathway.

Iodoacetate and *iodoacetamide*, inhibitors of sulfhydryl enzymes, block glycolysis at the glyceraldehyde 3-phosphate stage by irreversibly inhibiting *glyceraldehyde 3-phosphate dehydrogenase*. Iodoacetate or iodoacetamide binds covalently with and alkylate the SH group of the enzyme. It thus prevents the formation of the enzyme-substrate complex and catalysis. Addition of iodoacetate or iodoacetamide to a fermentation mixture leads to the accumulation of glyceraldehyde 3-phosphate; dihydroxyacetone phosphate and fructose 1,6-bisphosphate, normally glycolyzed through glyceraldehyde 3-phosphate, also get accumulated.



Fluoride blocks glycolysis at the 2-phosphoglycerate stage by inhibiting the Mg^{2+} -dependent enzyme *enolase*. Fluoride binds with Mg^{2+} to make it unavailable for enolase activity. Addition of fluoride to a fermentation mixture causes the accumulation of 2-phosphoglycerate; 3-phosphoglycerate, normally glycolyzed through 2-phosphoglycerate, also accumulates. Fluoride is frequently added to blood samples, meant for estimation of blood glucose, so as to prevent any loss of glucose due to glycolysis by blood cells.

Bromohydroxyacetone phosphate blocks glycolysis at the stage of dihydroxyacetone phosphate by irreversibly inhibiting *phosphotriose isomerase*. Because of its close structural similarity with the substrate dihydroxyacetone phosphate, the inhibitor binds covalently with the $\gamma\text{-COO}^-$ group of a glutamate residue at the active site of the enzyme to make it unavailable for catalysis. Addition of bromohydroxyacetone phosphate

to a fermentation mixture leads to the accumulation of dihydroxyacetone phosphate and fructose 1,6-bisphosphate.

Arsenate (AsO_4^{3-}) does not block glycolysis, but uncouples oxidation and phosphorylation at the step catalyzed by *glyceraldehyde 3-phosphate dehydrogenase*. Its close similarity to inorganic phosphate (PO_4^{3-}) enables arsenate to replace P_i in the cleavage of the enzyme-thioester intermediate, resulting in the production of highly labile 1-arseno-3-phospho-glycerate instead of 1,3-diphosphoglycerate. 1-Arseno-3-phosphoglycerate is hydrolyzed spontaneously into 3-phosphoglycerate without the formation of any ATP. Thus, addition of arsenate reduces the number of high-energy bonds generated by glycolysis.

Oxamate competitively inhibits lactate dehydrogenase to prevent the reoxidation of cytoplasmic NADH through the transfer of its electrons to pyruvate.

Regulation :

Phosphofructokinase is the enzyme for the rate-limiting step of glycolysis. Physiological concentrations ($\approx 5 \text{ mM litre}^{-1}$) of ATP in resting muscles inhibit phosphofructokinase allosterically and reduce its substrate-affinity for fructose 6-phosphate considerably. A 20% fall in ATP concentration raises the rate of glycolysis about tenfold and the phosphofructokinase activity by about 50%. Physiological concentrations of AMP oppose the negative allosteric effect of ATP. The ATP/AMP ratio is, therefore, significant in controlling muscle phosphofructokinase activity; a rise in this ratio decreases and a fall increases phosphofructokinase activity, with corresponding changes in the rate of glycolysis.

Fructose 6-phosphate (substrate), fructose 1,6-bisphosphate (product) and inorganic phosphate activate phosphofructokinase allosterically by counteracting its ATP-mediated allosteric inhibition and enhance

the activation of the enzyme in response to a fall in ATP/AMP ratio. But citrate allosterically inhibits phosphofructokinase and intensifies the ATP-mediated inhibition of the latter.

Increased glycolysis or β -oxidation, followed by the entry of their products into the TCA cycle, enhances the concentrations of citrate and ATP, and raises the ATP/AMP ratio. All these inhibit phosphofructokinase; fructose 6-phosphate and glucose 6-phosphate accumulate in consequence and *hexokinase* is allosterically inhibited by glucose 6-phosphate, stopping further glycolysis.

Glycogen phosphorylase exists in two forms in muscles: (i) *Phosphorylase b* is inactive except at high concentrations of AMP and P_i , and carries no phosphate group on its serine residues. (ii) *Phosphorylase a* is active even in absence of AMP and requires AMP only at low concentrations of P_i , but carries a phosphoserine residue in each of its peptide subunits. *Phosphorylase b* is changed to *phosphorylase a* through the phosphorylation of the serine residues by ATP and *phosphorylase kinase*, immediately followed by the joining of two phosphorylase molecules into one (Fig. 11.16). *Phosphorylase kinase*, in its turn, is made of four molecules each of α , β , γ and δ peptide subunits. The δ subunits consist of the Ca^{2+} -binding protein *calmodulin*; α and β subunits possess serine residues which remain phosphorylated in the *a* form of the enzyme and dephosphorylated in its *b* form. The inactive *phosphorylase kinase b* may be activated by the binding of Ca^{2+} with its *calmodulin* subunits. The *b* form may alternatively be converted to the active *phosphorylase kinase a* by the phosphorylation of serine residues in its α and β subunits by a *cAMP-dependent protein kinase*; this phosphorylation increases the Ca^{2+} -sensitivity of the δ subunit of *phosphorylase kinase a* which is fully activated only in presence

of phosphorylase *b* to phosphorylase *a* to enhance glycolysis.

During *muscle contraction*, the rise in Ca^{2+} concentration in the sarcoplasm leads to the binding of Ca^{2+} ions with calmodulin (δ) subunits of phosphorylase kinase *b*, changing its inactive *T* conformation to an active *R* conformation. Ca^{2+} binds also with the muscle protein called *troponin C* which closely resembles calmodulin. The Ca^{2+} -troponin complex can then enhance phosphorylase kinase *b* activity further. The activated phosphorylase kinase then phosphorylates and activates phosphorylase *b* to phosphorylase *a* to increase muscle glycolysis.

Insulin enhances glycolysis by inducing the synthesis of phosphofructokinase as well as pyruvate kinase, by enhancing the gene transcription of their mRNAs. Insulin also increases the activity of pyruvate kinase by increasing its dephosphorylation.

Pasteur effect :

This is the fall in the lactate production rate in aerobic cells in presence of molecular O_2 and results from the following :
(a) Aerobic metabolism through the TCA cycle produces large amounts of ATP and also citrate as an intermediate. Both ATP and citrate allosterically inhibit phosphofructokinase to reduce glycolysis. (b) Aerobic

metabolism uses up large amounts of ADP and P_i in oxidative phosphorylation and reduces their availability for glycolysis.
(c) The fall in ADP and P_i concentrations depresses the actions of phosphoglycerate kinase and pyruvate kinase, both having relatively high K_m values for P_i and ADP.
(d) The fall in P_i concentration amplifies the allosteric inhibition of phosphofructokinase by ATP.

Crabtree effect :

This is the fall in tissue respiration due to a rise in glucose concentration. High glucose levels enhance glycolysis which consumes ADP and P_i , reducing their availability for oxidative phosphorylation. This results in a reduction in the TCA cycle and mitochondrial respiration.

Microbial fermentations

Lactobacilli ferment glucose, lactose and sucrose to lactic acid through steps identical with those of muscle glycolysis.

Saccharomyces (yeast) produce ethanol and small amounts of glycerol and other products by the anaerobic fermentation of glucose and maltose. Maltose has to be hydrolyzed first to glucose by *maltase*. Glucose is fermented to pyruvate through the steps of glycolysis. But yeast lacks lactate dehydrogenase and instead

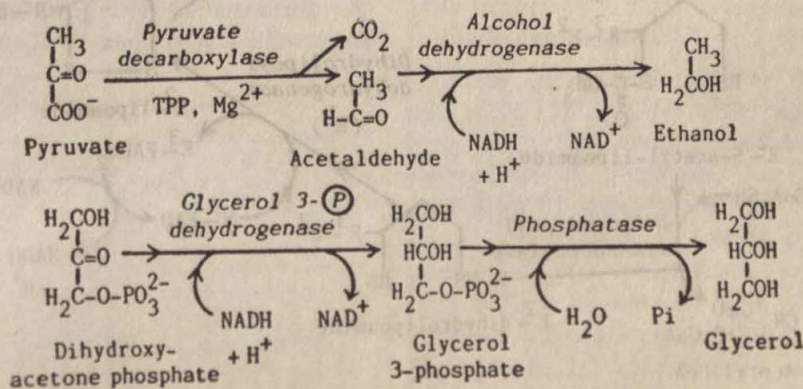


Fig. 11.17. Final reactions in yeast fermentation.

possesses pyruvate decarboxylase and alcohol dehydrogenase. So, instead of being reduced by NADH to lactate, pyruvate is decarboxylated to acetaldehyde by *pyruvate decarboxylase* (carboxylase) with the help of TPP (Fig. 11.17). Zn^{2+} -containing *alcohol dehydrogenase* then transfers reducing equivalents to acetaldehyde from NADH, generated by the earlier reaction catalyzed by *glyceraldehyde 3-phosphate dehydrogenase*; acetaldehyde is thereby reduced to ethanol. Before sufficient acetaldehyde has been formed *glycerol 3-phosphate dehydrogenase* may transfer reducing equivalents from NADH to dihydroxyacetone phosphate, producing *glycerol 3-phosphate*; the latter is hydrolyzed by a *phosphatase* into *glycerol*.

Acetic acid bacteria ferment glucose to acetaldehyde just like yeast, but oxidize acetaldehyde to acetic acid.

11.4 AEROBIC METABOLISM AND TCA CYCLE

Aerobic metabolism of carbohydrates is carried out in two phases. Pyruvate produced by glycolysis is first oxidized and decarboxylated to acetyl-CoA by the pyruvate dehydrogenase system. Acetyl-CoA is then oxidized in the citric acid cycle (TCA cycle).

Pyruvate dehydrogenase system

In presence of O_2 , lactate produced by glycolysis is reoxidized to pyruvate by lactate dehydrogenase and NAD^+ in the cytosol. A *pyruvate carrier* of the inner mitochondrial membrane then transports pyruvate from the cytosol to the mitochondrial matrix either in exchange of OH^- ions, or by *symport* (see page 126) utilizing the inward H^+ gradient across the membrane.

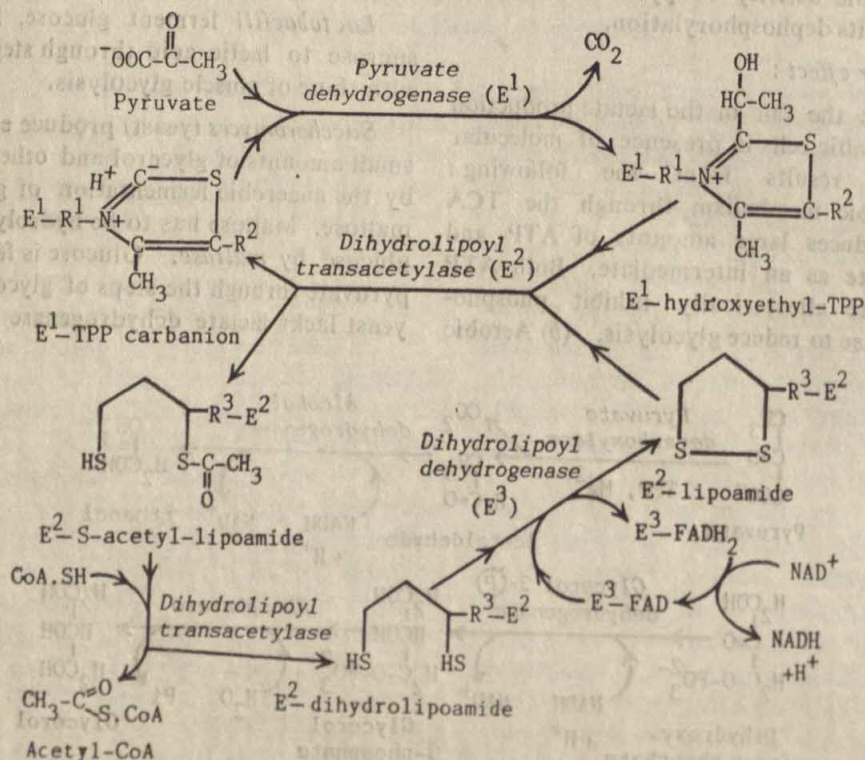
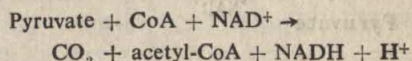


Fig. 11.18. Mode of action of pyruvate dehydrogenase system.

In the mitochondrial matrix, pyruvate is oxidatively decarboxylated to acetyl-CoA by a multienzyme system called *pyruvate dehydrogenase system*. The latter is constituted by the spontaneous assembly and noncovalent linking of the polypeptide chains of three enzymic components. (i) The *pyruvate dehydrogenase* component carries TPP as its prosthetic group. The carbonyl (C=O) group of pyruvate binds with the carbanion (C⁻) of the enzyme-bound TPP. The pyruvyl group is then decarboxylated to a hydroxyethyl group (Fig. 11.18). (ii) The *dihydrolipoyl transacetylase* component is the core of the multienzyme complex and carries α -lipoic acid as its prosthetic group; α -lipoic acid remains covalently bound to the ϵ -NH₂ of a lysine residue of the transacetylase—this α -lipoate-lysine combination is called *lipoamide* (Fig. 11.19). α -Lipoate ring is thus held at the end of a 1.4 nm long flexible arm formed by the linking of the C₆ sidechain of α -lipoate with the ϵ -NH₂ at the end of the C₄ sidechain of lysine. This enables the lipoate to carry the active intermediates between the active sites of different components of the system. The TPP-bound hydroxyethyl group is oxidized to an acetyl group by a transfer of its reducing equivalents (H⁺ and *e*) to α -lipoate and is simultaneously transferred to the reduced lipoate (dihydrolipoate) to form S-acetyl-lipoamide. The latter then transfers its acetyl group to coenzyme A, forming acetyl-CoA and dihydrolipoamide

(lysine-bound dihydrolipoate). (iii) The *dihydrolipoyl dehydrogenase* component carries FAD as its prosthetic group which accepts reducing equivalents from dihydrolipoamide, reoxidizing the latter to lipoamide and getting itself reduced to FADH₂. The latter is reoxidized to FAD by transferring its reducing equivalents to NAD⁺. The overall reaction is irreversible ($\Delta G^{\circ} \approx -8 \text{ kcal mol}^{-1}$):



The NADH is immediately reoxidized to NAD⁺ with the help of the mitochondrial respiratory chain and molecular O₂, leading to the formation of three high-energy phosphate bonds through oxidative phosphorylation. Pyruvate dehydrogenase is inhibited by arsenite.

Such multienzyme systems (other examples: α -ketoglutarate dehydrogenase, fatty acid synthase and glycine cleavage enzyme) enhance the rate and efficiency of the overall reaction, because (i) different enzymic components are closely held together enabling well-coordinated and sequential catalysis, (ii) the intermediates, instead of diffusing randomly to active sites of enzymes, are held on a long flexible arm of the enzyme, which transfers them directly to successive active sites, prevents their dilution by diffusion in the medium and also prevents their diversion to competing side reactions.

Citric acid cycle

Krebs' citric acid cycle or tricarboxylic acid cycle (*TCA cycle*) is the final common path for the aerobic oxidation of the endproducts of glycolysis, β -oxidation and catabolism of many amino acids. TCA cycle also serves anabolic functions by providing precursors for porphyrins, amino acids, fatty acids and steroids. The cycle operates in the mitochondrial matrix and is inoperative in erythrocytes devoid of mitochondria. It is highly operative in cardiac muscle,

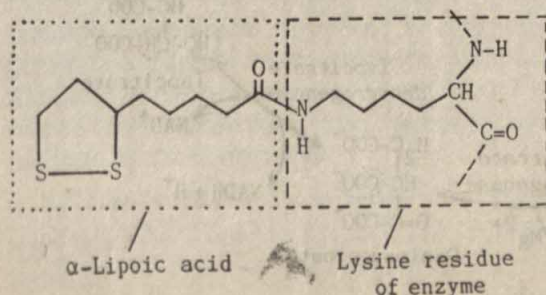


Fig. 11.19. Lipoamide.

liver and red striated muscle fibres, but is less operative in white striated muscle fibres which are mainly glycolytic. TCA cycle

operates in the brain also; thiamin deficiency produces polyneuritis partly through a failure of this pathway in the nerve tissue.

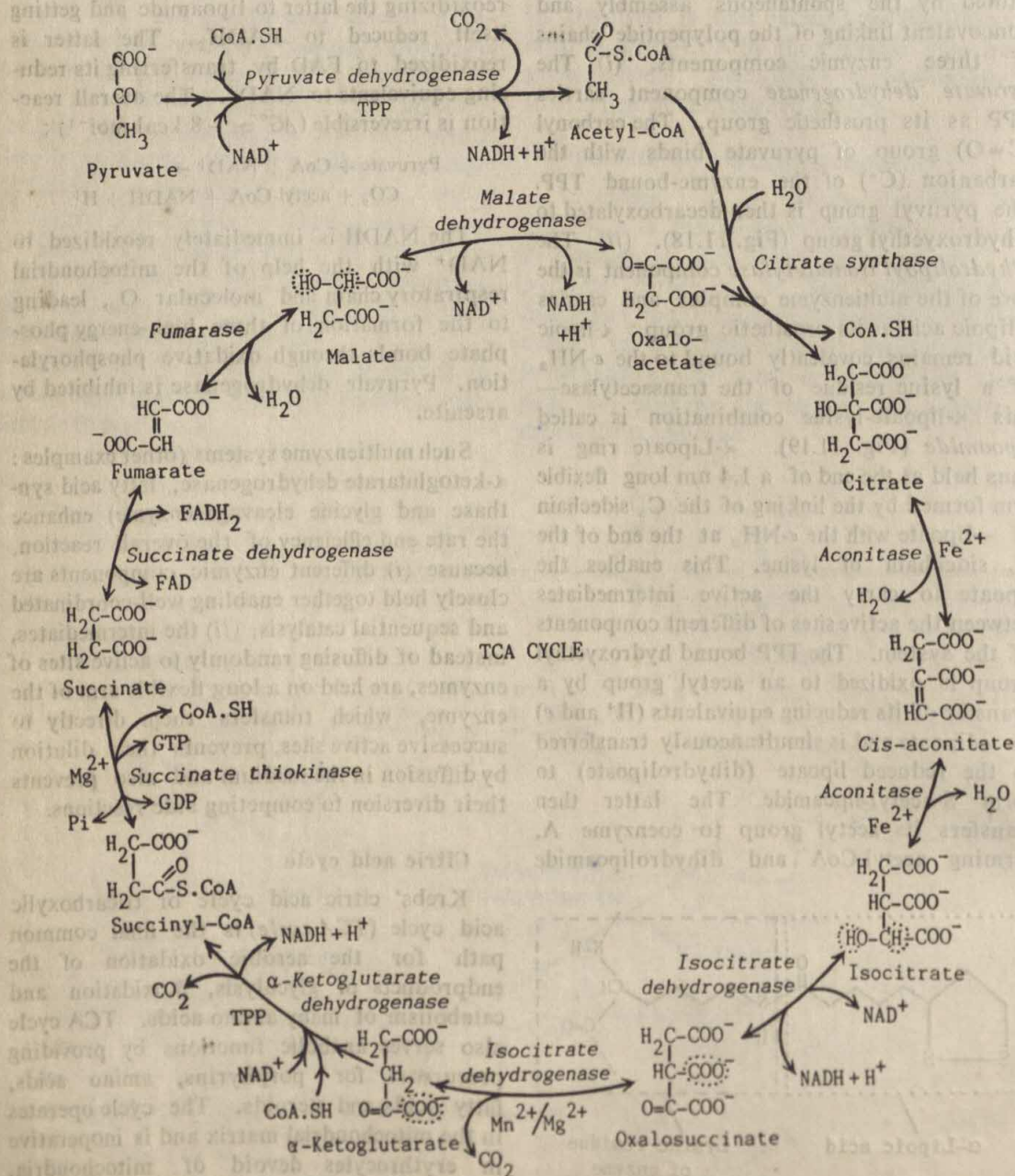


Fig. 11.20. TCA cycle.

Reaction sequence :

In this cycle, an acetyl (C_2) unit from acetyl-CoA condenses with oxaloacetate (C_4) to form citrate (C_6) which is systematically oxidized and decarboxylated by mitochondrial enzymes to regenerate oxaloacetate finally. Thus, each turn of the cycle changes a C_2 unit into CO_2 and H_2O . In addition to acetyl-CoA produced from pyruvate, fatty acids, ketone bodies, leucine and isoleucine, amino acid metabolites such as α -ketoglutarate and oxaloacetate can also enter the cycle at different steps.

There are four steps of oxidation in the cycle, each yielding high-energy phosphate bonds through *oxidative phosphorylation*. Another step of the cycle forms one high-energy phosphate bond through *substrate-level phosphorylation*.

(a) *Citrate synthase* condenses acetyl-CoA with oxaloacetate by aldol condensation, linking the methyl-C of acetyl-CoA with the ketonyl-C of oxaloacetate. An enzyme-citryl-CoA intermediate is thus formed. The thioester bond in citryl-CoA is immediately hydrolyzed to release citrate and coenzyme A (Fig. 11.20). The reaction is irreversible. ($\Delta G^\circ = -7.7 \text{ kcal mol}^{-1}$).

(b) *Aconitase*, an Fe^{2+} -enzyme, isomerizes citrate to isocitrate. It first dehydrates citrate to *cis*-aconitate by removing H and OH groups from its carbons derived respectively from the methylene-C and the ketonyl-C of oxaloacetate. Next, the enzyme hydrates *cis*-aconitate to isocitrate by adding H and OH groups in a reverse order to those carbons. Aconitase possesses *cis-trans* specificity; it acts on *cis*-aconitate only and is competitively inhibited by *trans*-aconitate.

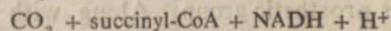
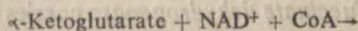
(c) *Isocitrate dehydrogenase* changes isocitrate (C_6) to α -ketoglutarate (C_5) in mitochondria. First, it oxidizes isocitrate to oxalosuccinate, using NAD^+ as the acceptor of a hydride (H^-) ion from isocitrate; this is

the first oxidative step of the TCA cycle. The NADH produced is reoxidized to NAD^+ by the mitochondrial electron transport chain, generating three high-energy phosphate bonds through oxidative phosphorylation. Next, the enzyme decarboxylates oxalosuccinate to α -ketoglutarate in presence of Mn^{2+} .

Cytoplasmic isocitrate dehydrogenases utilize $NADP^+$ instead of NAD^+ to catalyze a similar reaction and produce NADPH for serving in the reductive synthesis of fatty acids.

(d) *α -Ketoglutarate dehydrogenase*, a multienzyme system analogous to pyruvate dehydrogenase, irreversibly catalyzes the oxidative decarboxylation of α -ketoglutarate to succinyl-CoA; this is the second oxidative step of the TCA cycle ($\Delta G^\circ = -7.2 \text{ kcal mol}^{-1}$). The system has three components.

(i) *α -Ketoglutarate dehydrogenase* component carries TPP as the prosthetic group and decarboxylates α -ketoglutarate to an α -hydroxy- β -carboxypropyl group (Fig. 11.21). (ii) *Dihydrolipoyl transsuccinylase* contains lipoamide which is reduced while oxidizing the α -hydroxy- β -carboxypropyl group to succinyl group, and then receives the latter to form S-succinyl-lipoamide. The latter then transfers its succinyl group to coenzyme A forming succinyl-CoA and is itself changed to dihydrolipoamide. (iii) *Dihydrolipoyl dehydrogenase*, carrying FAD as its prosthetic group, reoxidizes dihydrolipoamide to lipoamide while FAD is reduced to $FADH_2$. The latter is reoxidized to FAD with the help of NAD^+ . The NADH produced thereby is reoxidized to NAD^+ by the mitochondrial electron transport chain, forming three high-energy phosphate bonds through oxidative phosphorylation. The overall reaction is as follows:



The enzyme system is inhibited by arsenite,

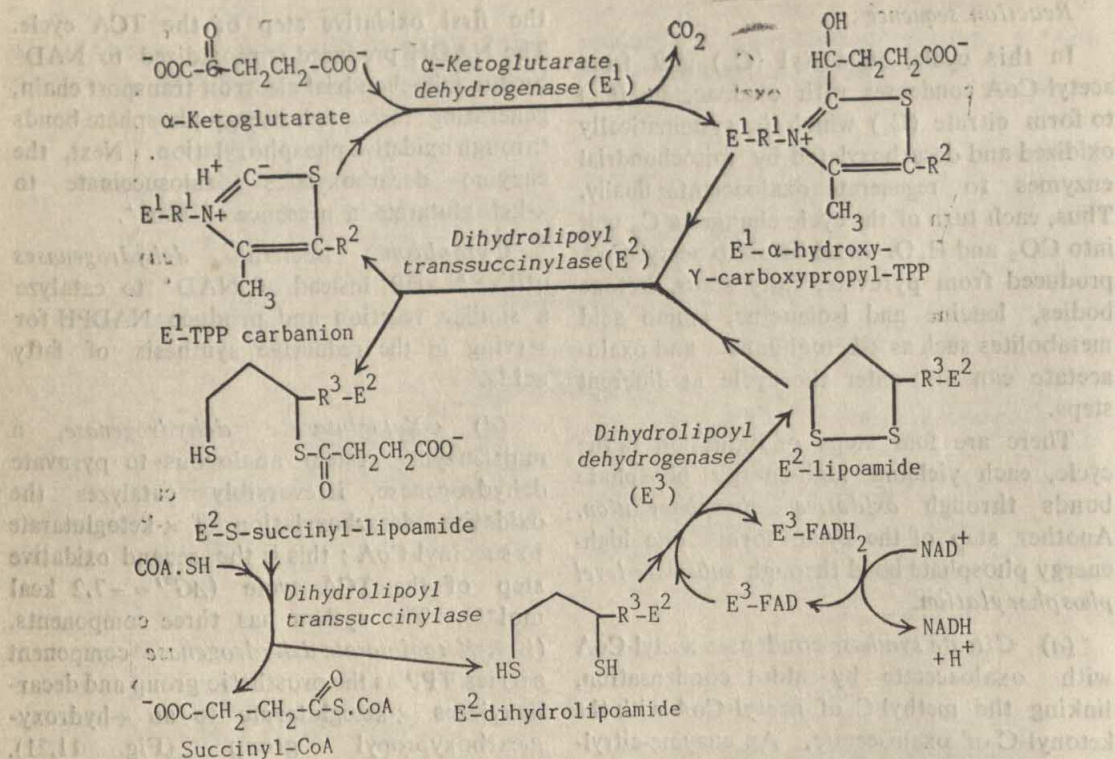
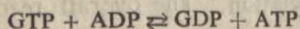


Fig. 11.21. Mode of action of α -ketoglutarate dehydrogenase system.

(e) *Succinate thiokinase* catalyzes the cleavage of the high-energy thioester bond of succinyl-CoA to produce succinate and coenzyme A, and utilizes the energy released in this exergonic reaction ($\Delta G^{\circ} = -8 \text{ kcal mol}^{-1}$) to drive the endergonic reaction of phosphorylation of GDP with Pi, forming GTP. Thus, a high-energy phosphate bond is produced at this step by substrate-level phosphorylation. (Overall $\Delta G^{\circ} = -0.8 \text{ kcal mol}^{-1}$.)

GTP may donate its high-energy phosphate bond in forming ATP from ADP under the action of *nucleoside diphosphokinase*.



(f) *Succinate dehydrogenase* carries FAD as its prosthetic group. Along with an iron-sulfur protein, it constitutes the *succinate-Q reductase complex* of the mitochondrial

respiratory chain (see page 296). Succinate dehydrogenase transfers reducing equivalents (H^+ and electrons) from succinate to FAD, reducing the latter to FADH₂ and oxidizing succinate to fumarate. This is the third oxidative reaction of the TCA cycle. FADH₂ is immediately reoxidized by a transfer of its electrons to the mitochondrial electron transport chain through the FeS protein and coenzyme Q. This leads to the formation of two high-energy phosphate bonds through oxidative phosphorylation. Succinate dehydrogenase shows a *cis-trans* specificity in producing only the *trans* isomer fumarate and not its *cis* isomer maleate.

(g) *Fumarase* hydrates fumarate into L-malate. The enzyme hydrates only the *trans* isomer fumarate and not its *cis* isomer maleate (*cis-trans* specificity); moreover, the product

TABLE 11.2. *High-energy bonds from aerobic metabolism of carbohydrates.*

Reaction	Enzyme	Mode of phosphorylation	High-energy bonds formed	
			per C_3	per C_6
Pyruvate $\rightarrow$ acetyl-CoA	Pyruvate dehydrogenase	Oxidative	3	6
<i>Citric acid cycle :</i>				
Isocitrate $\rightarrow$ α -ketoglutarate	Isocitrate dehydrogenase	Oxidative	3	6
α -Ketoglutarate $\rightarrow$ succinyl-CoA	α -Ketoglutarate dehydrogenase	Oxidative	3	6
Succinyl-CoA $\rightarrow$ succinate	Succinate thiokinase	Substrate-level	1	2
Succinate $\rightarrow$ fumarate	Succinate dehydrogenase	Oxidative	2	4
Malate $\rightarrow$ oxaloacetate	Malate dehydrogenase	Oxidative	3	6
Total			15	30

(i.e., the substrate for the reverse reaction) is always L-malate and not D-malate (D-L *stereospecificity*).

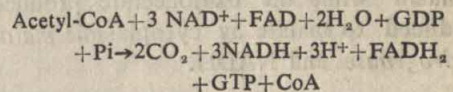
(h) *Malate dehydrogenase*, a sulphydryl enzyme of the mitochondrial matrix, oxidizes malate to oxaloacetate using NAD^+ as the electron-acceptor. This is the fourth oxidative step of the TCA cycle. NADH produced in this reaction is reoxidized by the mitochondrial electron transport chain, yielding three high-energy phosphate bonds through oxidative phosphorylation.

Formation of oxaloacetate completes one turn of the TCA cycle. Using oxaloacetate labelled with carbon radioisotopes, it has been shown that CO_2 evolved in the cycle comes entirely from the oxaloacetate-derived part of citrate and not from its acetate-derived part. So, oxaloacetate, yielded at the end of a turn of the cycle, always inherits two of its carbons from acetate which entered the cycle at the start of that turn. In other words, citrate is metabolized asymmetrically in the TCA cycle. This happens because *aconitase* can distinguish between the two CH_2COO^- groups in citrate as they are sterically nonequivalent, being bonded to its middle carbon bearing two different substituents, viz., OH and COO^- .

The *cytoplasmic isoenzyme of malate dehydrogenase*, instead of oxidizing malate, uses NADH to reduce oxaloacetate to malate; the latter is either used by *malic enzyme* for

reducing cytoplasmic $NADP^+$ to NADPH, or *shuttled* into mitochondria for transferring its reducing equivalents to mitochondrial NAD^+ (see page 300).

The stoichiometry of the TCA cycle is :



Energetics :

The aerobic metabolism of a pyruvate (C_3) molecule yields 15 high-energy phosphate bonds, three from the oxidative decarboxylation of pyruvate by pyruvate dehydrogenase and twelve from the TCA cycle steps (Table 11.2). These have already been elaborated above. The succinate dehydrogenase-catalyzed step yields only two high-energy bonds per pair of electrons transferred to the electron transport chain because these electrons pass from FAD to coenzyme Q directly, bypassing the NADH-Q reductase complex (see page 297).

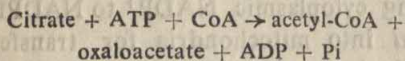
The aerobic metabolism of the glycolytic endproducts of a hexose (C_6) molecule yields 2×15 or 30 high-energy bonds.

Anabolic roles of TCA cycle :

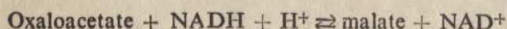
The citric acid cycle yields intermediates which are used in the following synthetic reactions.

(a) *Porphyrim synthesis*: Succinyl-CoA is used in the synthesis of δ -aminolevulinic acid (ALA) from which porphyrins are synthesized.

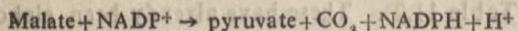
(b) *Fatty acid synthesis*: A tricarboxylate transporter of the inner mitochondrial membrane transfers citrate from the mitochondrial matrix to the cytosol in the liver, adipose tissue and lactating mammary glands. There citrate is cleaved into acetyl-CoA and oxaloacetate with the help of *ATP-citrate lyase* (citrate cleavage enzyme), ATP and coenzyme A.



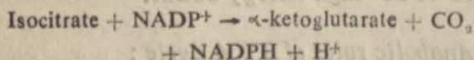
The acetyl-CoA thus produced is then used in the *de novo* synthesis of fatty acids in the cytoplasm while the oxaloacetate produced is reduced to malate by cytoplasmic *malate dehydrogenase* and NADH.



Malate is then oxidized and decarboxylated by *malic enzyme* (NADP-malate dehydrogenase), producing pyruvate and reducing NADP⁺ to NADPH which acts as a donor of reducing equivalents for fatty acid synthesis.



Particularly in ruminant animals, some citrate is isomerized to isocitrate by the cytoplasmic isozyme of *aconitase*. Isocitrate is then oxidized and decarboxylated to α -ketoglutarate by the cytoplasmic isozyme of *isocitrate dehydrogenase* with the simultaneous reduction of NADP⁺ to NADPH which is utilized as a donor of reducing equivalents for fatty acid synthesis.



In these ways, TCA cycle intermediates participate in fatty acid synthesis. ATP-citrate lyase is induced by high-carbohydrate

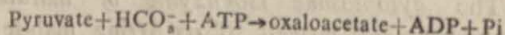
low-fat diets, insulin and thyroxine, all of which enhance fatty acid synthesis. Fasting, diabetes, obesity and high-fat diets depress that enzyme to reduce lipogenesis. ATP-citrate lyase and malic enzyme have poor activities in ruminants.

(c) *Gluconeogenesis*: Malate and oxaloacetate are changed to glucose by the process of gluconeogenesis in the cytoplasm. So, all TCA cycle intermediates as well as pyruvate can give rise to glucose through their conversion to malate or oxaloacetate. Many amino acids such as alanine, glutamate and aspartate are changed by transamination or deamination to pyruvate or TCA cycle intermediates which are in turn changed to glucose.

(d) *Amino acid synthesis*: α -Ketoglutarate and oxaloacetate produced in the TCA cycle may be used in the synthesis of some amino acids such as glutamate and aspartate.

Anaplerosis:

TCA cycle intermediates are removed by various anabolic and catabolic reactions. Oxaloacetate, for example, is continually used up by gluconeogenesis or by transamination to aspartate, α -ketoglutarate is removed by transamination to glutamate, citrate is transferred to the cytoplasm for giving acetyl-CoA there, and succinate is used up in heme synthesis. Anaplerosis consists of reactions for replenishing the TCA cycle intermediates to keep the cycle operative. The most important anaplerotic reaction is the carboxylation of pyruvate to oxaloacetate in mitochondria with the help of *pyruvate carboxylase*, ATP and biotin. As acetyl-CoA allosterically activates pyruvate carboxylase, accumulation of acetyl-CoA promotes anaplerosis.



Formation of α -ketoglutarate from glutamate either by *transaminases* or by *mitochon-*

drial L-glutamate dehydrogenase, may serve as another anaplerotic reaction.

Inhibitors :

Different inhibitors have been used with preparations of animal tissues such as muscles to study the course of the TCA cycle and the sequence of its steps.

Fluoroacetate inhibits aconitase and thereby blocks the TCA cycle to cause manifold accumulation of citrate in the tissues. Fluoroacetate occurs in the leaves of *Dichapetalum cymosum* and is used as a rat poison. Tissues thioesterify it to fluoroacetyl-CoA, and the fluoroacetyl group of the latter is next transferred to oxaloacetate to form fluorocitrate; fluorocitrate binds to aconitase by the chelation of its fluoride to the ferrous ion at the active site of the enzyme. This inhibits aconitase.

Trans-aconitate competitively inhibits aconitase which is stereospecific for *cis*-aconitate. This accumulates citrate in the tissue preparation.

Arsenite inhibits oxidative decarboxylation by α -ketoglutarate dehydrogenase and causes accumulation of α -ketoglutarate.

Malonate and **oxaloacetate** possess close structural similarities with succinate and consequently inhibit succinate dehydrogenase competitively. Poisoning with malonate, therefore, enhances K_m of this enzyme for succinate and blocks the TCA cycle by inhibiting the enzyme, causing accumulation of succinate.

Regulation

Pyruvate dehydrogenase :

Pyruvate dehydrogenase system (PDH) is regulated mainly by reversible covalent modifications through phosphorylation and dephosphorylation of its pyruvate dehydrogenase component (Fig. 11.22). The latter is inactivated by phosphorylation with the help of ATP and mitochondrial pyruvate

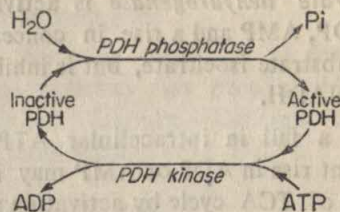


Fig. 11.22. Regulation of pyruvate dehydrogenase.

dehydrogenase kinase (PDH kinase), and is reactivated by dephosphorylation catalyzed by mitochondrial pyruvate dehydrogenase phosphatase (PDH phosphatase). A rise in ATP/ADP ratio, NADH/NAD⁺ ratio, acetyl-CoA concentration or cAMP concentration in the cell activates PDH kinase to enhance the phosphorylation and inactivation of PDH. Thus, in starvation and diabetes, acetyl-CoA and NADH produced by enhanced β -oxidation activate PDH kinase to inhibit PDH. A high-carbohydrate diet increases pyruvate through enhanced glycolysis; pyruvate increases PDH activity by inhibiting PDH kinase.

Insulin activates PDH phosphatase and consequently causes dephosphorylation and activation of PDH.

Citric acid cycle :

α -Ketoglutarate dehydrogenase is the enzyme for the rate-limiting step of TCA cycle and is regulated allosterically. It is inhibited by a rise in the ATP/ADP ratio, NADH/NAD⁺ ratio or succinyl-CoA/CoA ratio, all of which indicate a high energy-status of the cell; this results in a decline of the overall rate of TCA cycle. Ca²⁺, ADP or AMP activates the enzyme to enhance the operation of the cycle.

Citrate synthase is activated by the rise in the concentrations of its substrates, acetyl-CoA and oxaloacetate, and inhibited by the feed-back effect of its product citrate. It is also inhibited by ATP, NADH and long-chain fatty acyl-CoA like palmitoyl-CoA.

Isocitrate dehydrogenase is activated by Ca^{2+} , ADP, AMP and a rise in concentration of its substrate isocitrate, but is inhibited by ATP or NADH.

Thus, a fall in intracellular ATP and a consequent rise in ADP or AMP may increase the rate of TCA cycle by activating all three regulated enzymes of the cycle so as to replenish the ATP stock. With sufficient ATP and little ADP or AMP in the tissue, activities of these enzymes are inhibited and TCA cycle as well as ATP production declines.

11.5 GLYCOGENOSIS

Glycogenoses are *glycogen storage diseases* caused by the genetic deficiencies of different enzymes for glycogen metabolism. Of different types of glycogenosis, type VIII is inherited as a sex-linked recessive trait while others are inherited as autosomal recessive traits.

Type I. von Gierke's disease: This is caused by a genetic deficiency of *glucose 6-phosphatase* and a consequent failure to mobilize glycogen as blood glucose by glycogenolysis. Even adrenaline administration fails to increase glycogenolysis and the blood sugar level. Symptoms include excessive accumulation of glycogen in liver and kidney cells, severe hypoglycemia, ketosis, hyperlipemia and a massive hepatic enlargement.

Type II. Pompe's disease: This is caused by a genetic deficiency of *lysosomal α -1,4-glucosidase* which normally breaks down glycogen. Excessive amounts of glycogen accumulate in all tissues. Cardiac and respiratory failures cause death in infancy.

Type III. Cori's disease: This disease, also called *Forbe's disease* or *limit dextrinosis*, results from a genetic deficiency of *amylo-1,6-glucosidase (debranching enzyme)* which normally hydrolyzes the α -1,6 glycoside bonds at the branching points of the glycogen chain. The defect causes an accumulation of limit dextrin-type polysaccharide with short and

highly branched chains in muscles and liver due to incomplete glycogenolysis. Enlargement of liver and hypoglycemia are also seen.

Type IV. Andersen's disease: This is called *amylopectinosis*. It results from a deficiency of *amylo-1,4 $\rightarrow$ 1,6-transglucosidase* (branching enzyme). The inability to form α -1,6 glycosidic bonds during glycogenesis causes an abnormal amylose-like polysaccharide, with long and unbranched chains, to accumulate in the liver, muscles and spleen. Hepatic cirrhosis or cardiac failure causes death in infancy.

Type V. McArdle's disease: A genetic deficiency of *muscle glycogen phosphorylase* produces an inability for glycolysis in the muscle. This leads to a rise in muscle glycogen level, inability for heavy exercise, low blood lactate even after exercise, and severe muscle cramps.

Type VI. Her's disease: A genetic deficiency of *hepatic glycogen phosphorylase* results in a failure of hepatic glycogenolysis leading to high liver glycogen, hypoglycemia, ketosis, hepatic enlargement and failure of hyperglycemic response to adrenaline.

Type VII. Tarui's disease: An inherited deficiency of *phosphofructokinase* in muscles and erythrocytes results in a failure of glycolysis in those tissues, a rise in muscle glycogen, reduced capacity for muscular work, low blood lactate and muscle cramps.

Type VIII. Phosphorylase kinase deficiency: A genetic deficiency of *hepatic phosphorylase kinase* causes a failure of hepatic glycogen phosphorylase activity and a decrease in glycogenolysis. Mild hypoglycemia, rise in liver glycogen level and some hepatic enlargement are seen.

Type IX. Glycogen synthase deficiency: A genetic deficiency of *hepatic glycogen synthase* results in a failure of glycogenesis in the liver and a low liver glycogen level.

11.6 PENTOSE PHOSPHATE PATHWAY

This oxidative pathway, also called the *hexose monophosphate shunt* or the *phosphogluconate pathway*, operates in the *cytosol* of cells. It is very little active in highly glycolytic tissues such as skeletal muscles and non-lactating mammary glands, but is highly active in tissues such as the liver, adrenal cortex, testis, adipose tissue and lactating mammary glands where fatty acids, steroids or pentoses are synthesized.

The first part of this pathway constitutes its *oxidative phase* which oxidizes glucose 6-phosphate into ribulose 5-phosphate with the help of NADP^+ and reduces the latter to NADPH . This is followed by the *nonoxidative phase* which converts ribulose 5-phosphate to ribose 5-phosphate and also changes the latter and other C_5 and C_4 sugars into fructose 6-phosphate and glyceraldehyde 3-phosphate.

Reaction sequence

The following sequence of the pentose phosphate pathway has been established by the work of Warburg, Dickens, Lipmann, Horecker and Racker (Fig. 11.23).

1. Oxidative phase :

Enzymes of this phase require Ca^{2+} , Mg^{2+} or Mn^{2+} for activity.

(a) *Glucose 6-phosphate dehydrogenase* oxidizes glucose 6-phosphate irreversibly to 6-phosphogluconolactone with the help of NADP^+ which is simultaneously reduced to NADPH (Fig. 11.24).

(b) *Gluconolactone hydrolase* hydrolyzes 6-phosphogluconolactone irreversibly to 6-phosphogluconate.

(c) *6-Phosphogluconate dehydrogenase* oxidizes 6-phosphogluconate irreversibly to 3-keto-6-phosphogluconate, reducing NADP^+ to NADPH .

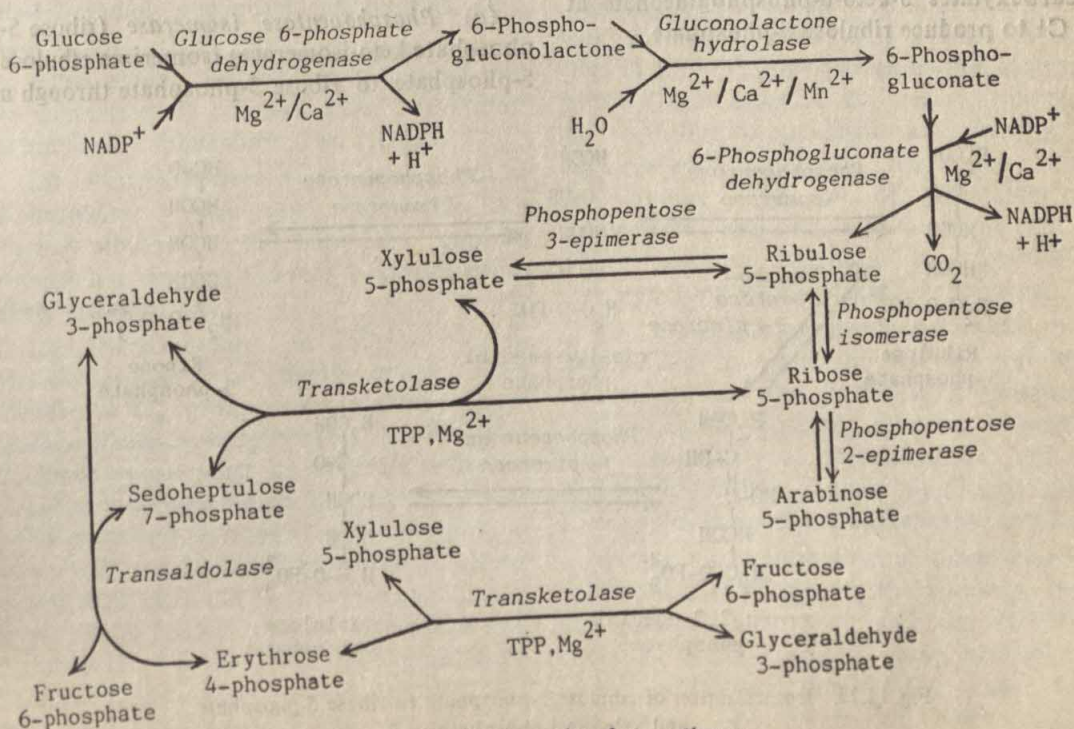


Fig. 11.23. Pentose phosphate pathway.

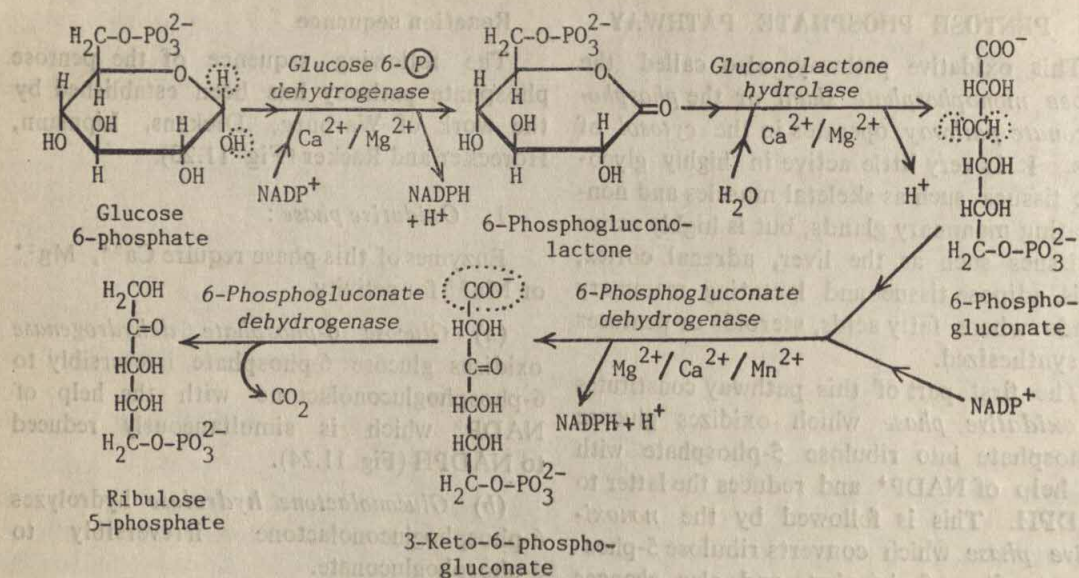


Fig. 11.24. Oxidative phase of pentose phosphate pathway.

simultaneously to NADPH. The enzyme then decarboxylates 3-keto-6-phosphogluconate at its C^1 to produce ribulose 5-phosphate.

2. Nonoxidative phase :

(a) **Phosphopentose isomerase** (ribose 5-phosphate keto-isomerase) isomerizes ribulose 5-phosphate to ribose 5-phosphate through a

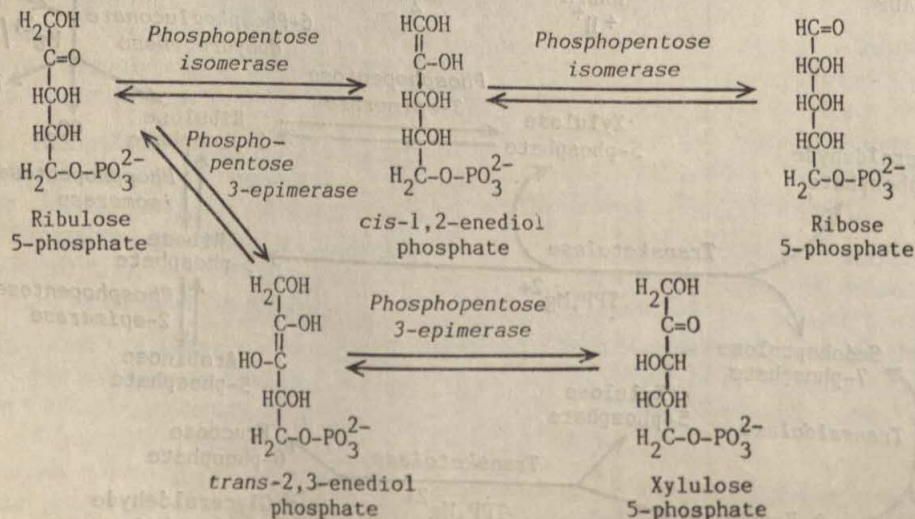


Fig. 11.25. Isomerization of ribulose 5-phosphate to ribose 5-phosphate and xylulose 5-phosphate.

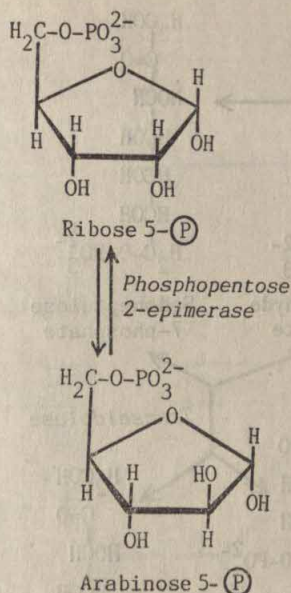


Fig. 11.26. Isomerization of ribose 5-phosphate to arabinose 5-phosphate.

cis-1,2-enediol phosphate intermediate (Fig. 11.25).

Some ribose 5-phosphate molecules may be isomerized by *phosphopentose 2-epimerase* to arabinose 5-phosphate (Fig. 11.26).

(b) *Phosphopentose 3-epimerase* (ribulose 5-phosphate 3-epimerase) side-by-side isomerizes ribulose 5-phosphate into xylulose 5-phosphate through a *trans*-2,3-enediol phosphate intermediate.

(c) *Transketolase*, an Mg^{2+} -requiring dimeric enzyme with tightly bound TPP, transfers a C_2 group from xylulose 5-phosphate to ribose 5-phosphate (Fig. 11.27). The C_2 -donor sugar must be a ketose with an L-configuration around its C^3 ; e.g., xylulose 5-phosphate and sedoheptulose 7-phosphate in respectively the forward and reverse reactions of this step. But the C_2 -acceptor sugar must be an aldose; e.g., ribose 5-phosphate and glyceraldehyde 3-phosphate in respectively the forward and reverse reactions of this step. The C^2 -ketonyl group of xylulose

5-phosphate first gets bound to the C^1 -carbanion of the enzyme-bound TPP, the C^2 - C^3 bond of the ketose is then cleaved to release a glyceraldehyde 3-phosphate molecule, leaving a glycolaldehyde unit on the TPP (pages 141-142 and Fig. 7.8). That unit is next transferred from TPP to the C^1 -carbonyl group of ribose 5-phosphate changing the latter to sedoheptulose 7-phosphate (C_7).

(d) *Transaldolase* transfers a three-carbon (C^1 - C^3) group from sedoheptulose 7-phosphate to the C^1 of glyceraldehyde 3-phosphate, changing them respectively to erythrose 4-phosphate (C_4) and fructose 6-phosphate (Fig. 11.23). The C_3 -donor is always a ketose; viz., sedoheptulose 7-phosphate and fructose 6-phosphate for respectively the forward and reverse reactions; the C_3 -acceptor is always an aldose, viz., glyceraldehyde 3-phosphate and erythrose 4-phosphate for the forward and reverse reactions respectively. The C^2 -ketonyl group of sedoheptulose 7-phosphate first forms a covalent Schiff base with the ϵ - NH_2 of a lysine of *transaldolase* (Fig. 11.28). This is followed by the cleavage of the C^3 - C^4 bond in the ketose, releasing an erythrose 4-phosphate and leaving a dihydroxyacetone unit in Schiff base with the enzyme. The C^1 of glyceraldehyde 3-phosphate then binds with the carbanion of dihydroxyacetone, the Schiff base is hydrolyzed and fructose 6-phosphate is released.

(e) *Transketolase* then transfers the two-carbon C^1 - C^2 group of still another xylulose 5-phosphate to the erythrose 4-phosphate produced in the preceding reaction, changing them respectively to glyceraldehyde 3-phosphate and fructose 6-phosphate (Fig. 11.27). It resembles the earlier *transketolase* reaction.

During the metabolism of three glucose 6-phosphate molecules in this pathway, they first produce three CO_2 and six NADPH molecules in the oxidative phase, and change in the subsequent nonoxidative phase into one ribose 5-phosphate and two xylulose

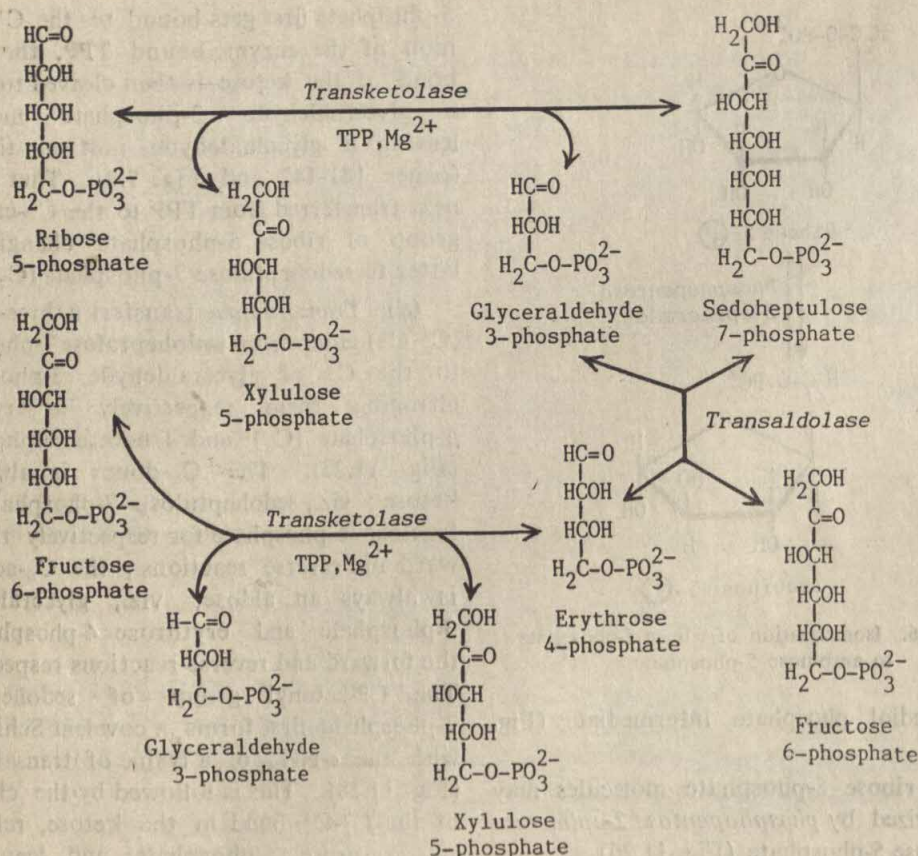


Fig. 11.27. Action of transketolase and transaldolase.

5-phosphate molecules whose interactions finally yield one glyceraldehyde 3-phosphate and two fructose 6-phosphate molecules (Fig. 11.23).

Genetic errors

Glucose 6-phosphate dehydrogenase deficiency: Genetically variant forms of glucose 6-phosphate dehydrogenase, inherited as a sex-linked trait, greatly reduce its activity in erythrocytes, decreasing the availability of NADPH for maintaining glutathione in the reduced state. (Glutathione destroys toxic peroxides and prevents oxidation of hemoglobin to methemoglobin.) This genetic disorder, relatively frequent among Parsis,

Punjabis, Sindhis and Kutchi-Lohanas, increases erythrocyte fragility, thereby causing massive hemolysis, anemia, jaundice, acute fall in blood hemoglobin level and blackening of urine. The symptoms are developed or aggravated after the intake of fava beans, vitamin K, pamaquine, sulfonamides and acetylsulfuric acid.

Wernicke-Korsakoff syndrome: A genetically variant form of transketolase considerably weakens the avidity of the enzyme for binding TPP. This makes the patient highly susceptible to thiamin deficiency which lowers the transketolase activity considerably to produce severe neuropsychiatric symptoms (page 141).

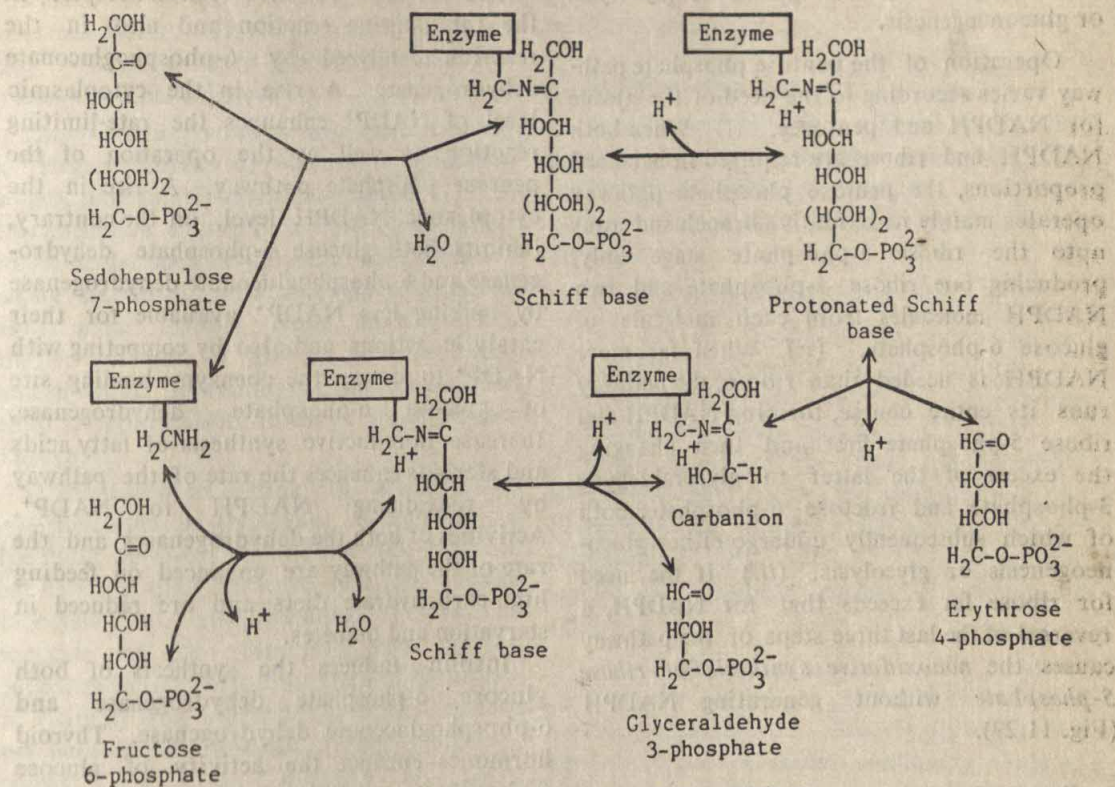


Fig. 11.28. Mode of action of transaldolase.

Significance

Unlike glycolysis and the TCA cycle, the pentose phosphate pathway utilizes NADP^+ instead of NAD^+ and makes very little contribution to ATP production because the reoxidation of NADPH by the mitochondrial respiratory chain is very limited.

(a) The pentose phosphate pathway generates NADPH which is utilized as an electron-donor in many reductive biosyntheses such as the cytoplasmic synthesis of fatty acids, the microsomal desaturation of fatty acids, the synthesis of cholesterol, steroids and sphingolipids, and the cytoplasmic synthesis of L-glutamate by L-glutamate dehydrogenase. The pathway is highly active in tissues carrying out such reductive syntheses in a large way.

(b) The reactions of this pathway are manifold increased in leucocytes during phagocytosis and the NADPH generated is utilized by NADPH oxidase in producing superoxide anions (O_2^-) for destroying phagocytosed materials. In erythrocytes, NADPH generated by this pathway is used by glutathione reductase in reducing oxidized glutathione.

(c) The pathway synthesizes ribose 5-phosphate which is used in incorporating ribose into nucleotides and nucleic acids.

(d) Arabinose 5-phosphate, produced from ribose 5-phosphate, is used for incorporating arabinose into glycoproteins.

(e) Pentoses such as ribose and xylulose are converted by this pathway to glyceraldehyde 3-phosphate and fructose 6-phosphate

and through them, undergo either glycolysis or gluconeogenesis.

Operation of the pentose phosphate pathway varies according to the need of the tissue for NADPH and pentoses. (i) When both NADPH and ribose are required in balanced proportions, the pentose phosphate pathway operates mainly its oxidative branch and runs upto the ribose 5-phosphate stage only, producing one ribose 5-phosphate and two NADPH molecules from each molecule of glucose 6-phosphate. (ii) When far more NADPH is needed than ribose, the pathway runs its entire course, forming NADPH and ribose 5-phosphate first and then changing the excess of the latter to glyceraldehyde 3-phosphate and fructose 6-phosphate, both of which subsequently undergo either gluconeogenesis or glycolysis. (iii) If the need for ribose far exceeds that for NADPH, a reversal of the last three steps of the pathway causes the *nonoxidative synthesis of ribose 5-phosphate* without generating NADPH (Fig. 11.29).

Regulation

The reaction, catalyzed by glucose 6-phosphate dehydrogenase, constitutes the *rate-limiting step* of the pentose phosphate pathway. It is primarily regulated by the cytoplasmic levels of NADP^+ and NADPH.

NADP^+ serves as the electron-acceptor in the rate-limiting reaction and also in the reaction catalyzed by 6-phosphogluconate dehydrogenase. A rise in the cytoplasmic level of NADP^+ enhances the rate-limiting reaction as well as the operation of the pentose phosphate pathway. A rise in the cytoplasmic NADPH level, on the contrary, inhibits both glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase by making less NADP^+ available for their catalytic actions and also by competing with NADP^+ to occupy the coenzyme-binding site of glucose 6-phosphate dehydrogenase. Increase in reductive synthesis of fatty acids and steroids enhances the rate of the pathway by reoxidizing NADPH to NADP^+ . Activities of both the dehydrogenases and the rate of the pathway are enhanced on feeding high-carbohydrate diets and are reduced in starvation and diabetes.

Insulin induces the synthesis of both glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Thyroid hormones enhance the activity of glucose 6-phosphate dehydrogenase. So, these hormones enhance the pentose phosphate pathway.

11.7 GLUCONEOGENESIS

Gluconeogenesis is the process of formation of glucose from non-carbohydrate

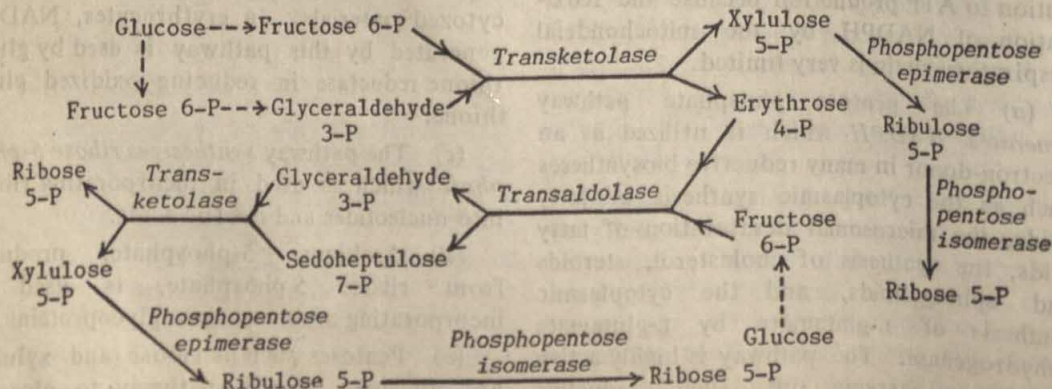


Fig. 11.29. Nonoxidative synthesis of ribose 5-phosphate.

materials. It is carried out in the *liver* and the *renal cortex*. The liver normally synthesizes 9-10 times more glucose by gluconeogenesis than the kidneys.

Lactate and *pyruvate* are quantitatively the largest sources of glucose in gluconeogenesis, particularly in intense exercise. Next in quantitative importance are the *glucogenic amino acids* such as glycine and alanine; during starvation, however, gluconeogenesis takes place mainly from amino acids. Smaller amounts of glucose are synthesized from *glycerol*. In ruminants, *propionate* from cellulose fermentation forms a major source of glucose.

Gluconeogenesis runs in a direction opposite to that of glycolysis. Some steps of glycolysis are reversible and the same enzymes that catalyze their forward reactions during glycolysis, can also catalyze the reverse reactions of the respective steps during gluconeogenesis. But three steps of glycolysis are virtually irreversible due to energy barriers and have to be bypassed during gluconeogenesis (Table 11.3). Enzymes for these irreversible gluconeogenic steps are often called *key gluconeogenic enzymes*. Of these four key enzymes, pyruvate carboxylase occurs in the mitochondrial matrix, phosphoenolpyruvate carboxykinase (PEPCK) in both mitochondria and cytosol of man, fructose 1,6-bisphosphatase in the cytosol only, and

glucose 6-phosphatase in the endoplasmic reticulum membrane.

Reaction sequence

Gluconeogenesis takes place in most cases partly in the mitochondrial matrix and partly in the cytosol; the very last step is carried out on the microsomal (endoplasmic reticulum) membrane.

1. Gluconeogenesis from lactate :

(a) Lactate produced by glycolysis is reoxidized to pyruvate in the cytosol by *lactate dehydrogenase* and NAD^+ , the latter being simultaneously reduced to NADH . A *pyruvate carrier* of the inner mitochondrial membrane then transports pyruvate from the cytosol to the mitochondrial matrix either in exchange of OH^- ions or by utilizing the inward H^+ gradient across the membrane (Fig. 11.30).

(b) In the mitochondrial matrix, *pyruvate carboxylase* condenses pyruvate with CO_2 to produce oxaloacetate. This key gluconeogenic enzyme is a *C—C ligase* containing biotin in its prosthetic group. It utilizes ATP and CO_2 to form an active enzyme-bound carboxybiotin intermediate from which the COO^- group is transferred to pyruvate (Fig. 7.22 and pages 154-155). Biotin thus acts as a carrier of the activated CO_2 for carboxylating pyruvate. Formation of the carboxybiotin

TABLE 11.3. Irreversible steps of glycolysis and the gluconeogenic enzymes for the reverse steps.

Glycolysis		Gluconeogenesis	
Irreversible reactions	Enzymes	Reverse reactions	Enzymes
(a) Phosphoenolpyruvate (PEP) $\rightarrow$ pyruvate	Pyruvate kinase	(a) Pyruvate $\rightarrow$ oxaloacetate Oxaloacetate $\rightarrow$ PEP	Pyruvate carboxylase PEPCK
(b) Fructose 6-P $\rightarrow$ fructose 1,6-bisphosphate	Phosphofructokinase	(b) Fructose 1,6-bisphosphate $\rightarrow$ fructose 6-P	Fructose 1-6 bisphosphatase
(c) Glucose $\rightarrow$ Glucose 6-P	Hexokinase	(c) Glucose 6-P $\rightarrow$ Glucose	Glucose 6-phosphatase

intermediate is facilitated by the binding of acetyl-CoA with the allosteric site of pyruvate carboxylase.

(c) Oxaloacetate is next reduced to malate by NADH and mitochondrial *malate dehydrogenase* of the TCA cycle. A *dicarboxylate transporter* of the inner mitochondrial membrane then transfers malate to the cytosol in exchange of HPO_4^{2-} ions.

(d) Cytoplasmic *malate dehydrogenase* reoxidizes malate to oxaloacetate in the cytosol, utilizing NAD^+ as the electron-acceptor and reducing the latter to NADH.

As an alternative to steps (c) and (d), oxaloacetate may be transaminated in the mitochondrion to aspartate which may be transported to the cytosol by an *aspartate-*

glutamate transporter of the inner membrane and reconverted to oxaloacetate by a *transaminase* in the cytosol (Fig. 10.13).

(e) In the cytosol, *phosphoenolpyruvate carboxykinase* (PEPCK) decarboxylates oxaloacetate and drives its phosphorylation to phosphoenolpyruvate (PEP) with the help of either GTP or ITP (Fig. 11.30).

When mitochondrial PEPCK is present, as in man, it changes oxaloacetate to PEP which is then transferred to the cytosol. In such cases, oxaloacetate need not be changed to malate in mitochondria and steps (c) to (e) are omitted.

(f) Glycolytic enzymes catalyze five successive reactions to convert PEP to fructose 1,6-bisphosphate (fructose 1,6-di-

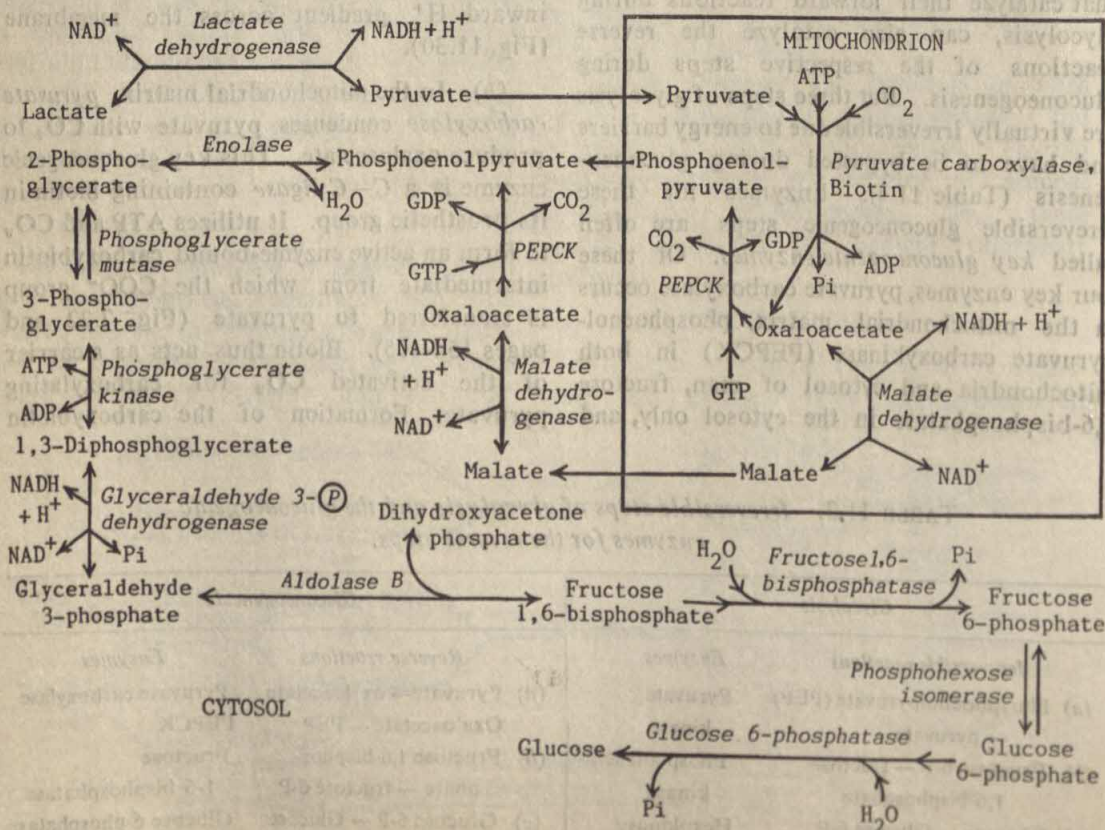


Fig. 11.30. Gluconeogenesis from lactate.

phosphate): (i) *Enolase* hydrates PEP to 2-phosphoglycerate. (ii) *Phosphoglycerate mutase* isomerizes the latter to 3-phosphoglycerate. (iii) *Phosphoglycerate kinase* uses ATP to phosphorylate 3-phosphoglycerate to 1,3-diphosphoglycerate. (iv) *Glyceraldehyde 3-phosphate dehydrogenase* reduces and dephosphorylates 1,3-diphosphoglycerate to produce glyceraldehyde 3-phosphate and P_i . For this reduction, the enzyme utilizes NADH as the electron donor. When lactate is undergoing gluconeogenesis, glyceraldehyde 3-phosphate dehydrogenase utilizes the NADH generated by lactate dehydrogenase in oxidizing lactate to pyruvate in step (a). But when pyruvate itself is undergoing gluconeogenesis, the electron donor is the NADH generated by malate dehydrogenase in oxidizing malate in step (d). (v) *Aldolase B* next condenses glyceraldehyde 3-phosphate with dihydroxyacetone phosphate by aldol condensation, producing fructose 1,6-bisphosphate.

(g) *Fructose 1,6-bisphosphatase*, another key gluconeogenic enzyme, hydrolyzes fructose 1,6-bisphosphate to fructose 6-phosphate and P_i . This enzyme occurs in the cytosol of hepatic and renal cells, but not in cardiac and smooth muscle cells. Though present in striated muscle fibres, its activity is far lower there.

(h) *Phosphohexose isomerase*, another glycolytic enzyme, isomerizes fructose 6-phosphate to glucose 6-phosphate.

(i) *Glucose 6-phosphatase* of hepatic microsomal membrane hydrolyzes glucose 6-phosphate to release glucose in the endoplasmic reticular lumen.

Energy expenditure: Six high-energy phosphate bonds are spent in the gluconeogenesis of one glucose molecule from two lactate or pyruvate molecules (Table 11.4).

TABLE 11.4. *High-energy phosphate bonds ($\sim P$) spent in gluconeogenesis.*

Reaction	$\sim P$ spent
Per lactate (C_3) molecule :	
pyruvate $\rightarrow$ oxaloacetate	1 (ATP)
oxaloacetate $\rightarrow$ phosphoenolpyruvate	1 (GTP)
3-phosphoglycerate $\rightarrow$ 1,3-diphosphoglycerate	1 (ATP)
Total	3
Total per glucose (C_6) molecule	6

2. Gluconeogenesis from glycerol :

(a) *Glycerokinase* uses ATP to phosphorylate glycerol to glycerol 3-phosphate in the hepatic and renal cytosol (Fig. 11.31).

(b) *Cytoplasmic glycerol 3-phosphate dehydrogenase* oxidizes glycerol 3-phosphate to dihydroxyacetone phosphate, using NAD^+ as the electron-acceptor.

(c) *Aldolase B* condenses dihydroxyacetone phosphate with glyceraldehyde 3-phosphate to form fructose 1,6-bisphosphate

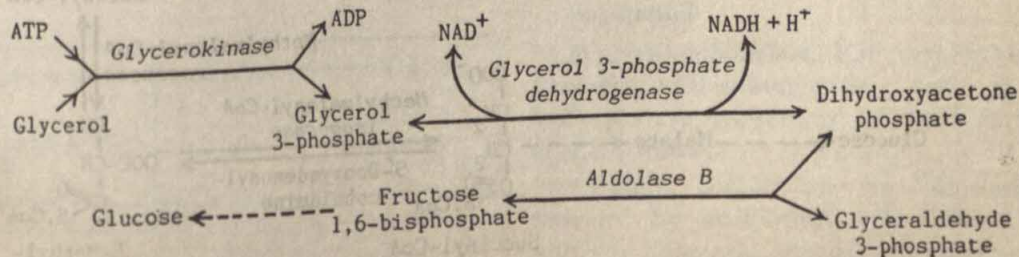


Fig. 11.31. Gluconeogenesis from glycerol.

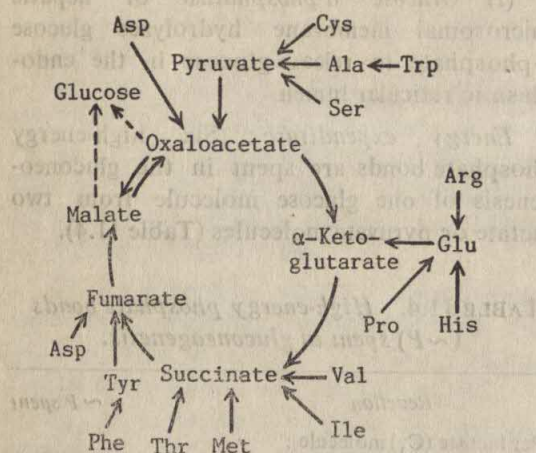


Fig. 11.32. Gluconeogenesis from amino acids.

(d) The latter is then changed to glucose through steps described for gluconeogenesis from lactate (Fig. 11.30).

3. Gluconeogenesis from amino acids :

On losing their amino groups, some amino acids form gluconeogenic intermediates ; e.g., α -ketoglutarate from glutamate, arginine, proline and histidine ; succinate from valine, methionine, isoleucine and threonine ; fumarate from aspartate and tyrosine. These

intermediates are converted to malate through the TCA cycle in mitochondria (Fig. 11.32). Pyruvate, formed from alanine, cysteine, serine and tryptophan, is carboxylated by pyruvate carboxylase to oxaloacetate ; oxaloacetate is also formed from aspartate. Oxaloacetate is then changed in mitochondria to either malate or phosphoenolpyruvate. Malate and PEP, after being transferred to the cytosol, may be changed to glucose (Fig. 11.30).

4. Gluconeogenesis from propionate :

(a) *Acetate thiokinase*, a C—S ligase, uses ATP and coenzyme A to thioesterify propionate to propionyl-CoA, through a propionyl-adenylate (propionyl-AMP) intermediate (Fig. 11.33).

(b) *Propionyl-CoA carboxylase*, a C—C ligase with biotin in its prosthetic group, carboxylates propionyl-CoA with CO_2 to form D-methylmalonyl-CoA. Like pyruvate carboxylase and acetyl-CoA carboxylase, the enzyme uses ATP and CO_2 to form carboxybiotin and transfers the COO^- group from the latter to the substrate.

(c) *Methylmalonyl-CoA racemase* of mitochondria isomerizes D-methylmalonyl-CoA to its L isomer.

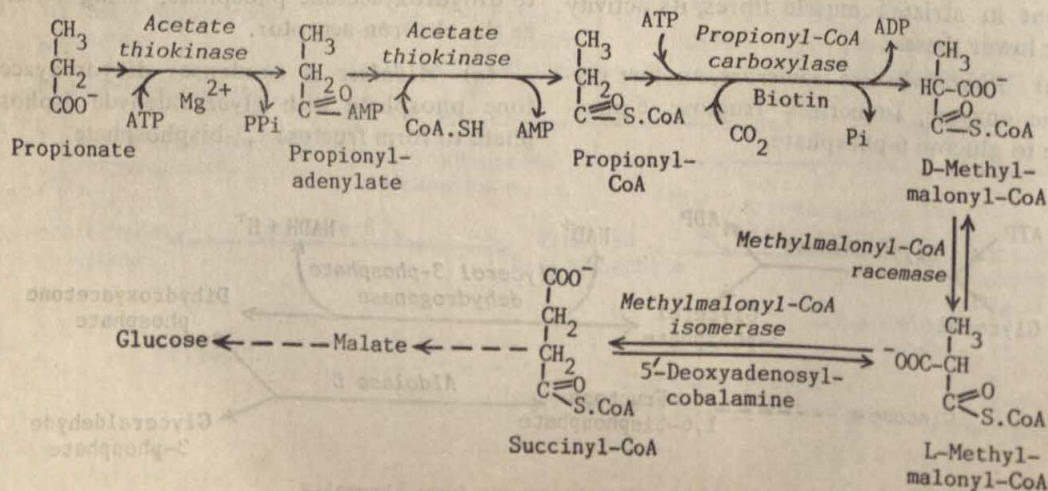


Fig. 11.33. Gluconeogenesis from propionate.

(d) *Methylmalonyl-CoA isomerase* (mutase), a mitochondrial enzyme containing 5'-deoxyadenosylcobalamin as its coenzyme, isomerizes L-methylmalonyl-CoA to succinyl-CoA.

(e) Succinyl-CoA is changed through the TCA cycle to malate. The latter is transferred to the cytosol where it is changed to glucose.

Methylmalonic aciduria: This inborn disease is characterized by about 200-fold rise in urinary methylmalonate, acidosis and a fall in blood pH to 7.0. It arises from a failure to convert methylmalonate to succinyl-CoA due to a genetic deficiency either of methylmalonyl-CoA isomerase, or of the adenosyltransferase for forming its coenzyme 5'-deoxyadenosylcobalamin from vitamin B₁₂. In the latter case but not in the former one, large parenteral doses of cobalamin restore the blood pH, decrease urinary methylmalonate and prolong the life.

Significance of gluconeogenesis

Gluconeogenesis serves several roles.

(a) It maintains the liver glycogen and a steady basal level of the blood sugar during starvation. After the first 48 hours of starvation, gluconeogenesis replaces glycogenolysis almost totally in adding glucose to blood.

Gluconeogenesis is thus of prime importance in starvation for maintaining the supply of glucose to the brain, kidneys, erythrocytes and muscles.

(b) It removes from blood the metabolites of glycolysis and lipolysis, and recycles them

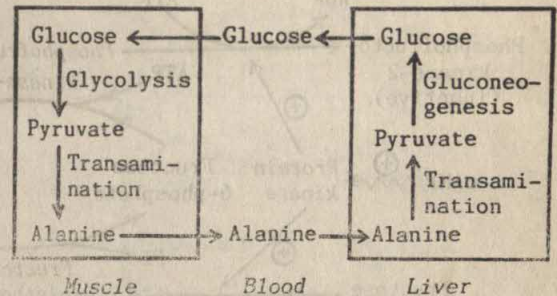


Fig. 11.35. Glucose-alanine cycle.

as carbohydrates. Lactate produced by glycolysis is largely released by muscles into the blood, carried to the liver, and reconverted by gluconeogenesis to glucose which is carried to muscles for glycolysis again (*Cori cycle*) (Fig. 11.34). Some pyruvate may be transaminated in muscles to alanine which is carried by blood to the liver and transaminated to pyruvate there (Fig. 11.35); gluconeogenesis converts pyruvate to glucose which is returned by blood to muscles (*glucose-alanine cycle*). Similarly, hepatic gluconeogenesis is a major fate of glycerol released by adipose tissue lipolysis.

(c) Gluconeogenesis provides the pathway for utilizing and metabolizing the carbon skeletons of gluconeogenic amino acids.

(d) It serves as the major metabolic pathway for propionate. Genetic failure of gluconeogenesis from propionate produces fatal *methylmalonic aciduria* in human infants. Gluconeogenesis from propionate is important in maintaining the blood sugar in ruminants.

Regulation

Pyruvate carboxylase, PEP carboxykinase, fructose 1,6-bisphosphatase and glucose 6-phosphatase are the key regulated enzymes of this pathway.

Pyruvate carboxylase is allosterically activated by acetyl-CoA. The latter binds with the allosteric site of the enzyme and alters its tertiary structure. This increases

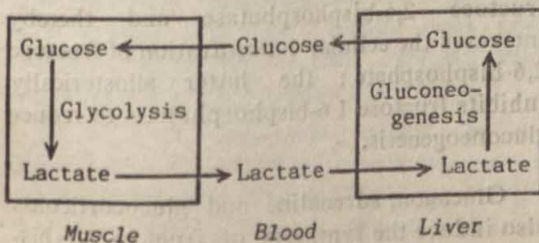


Fig. 11.34. Cori cycle.

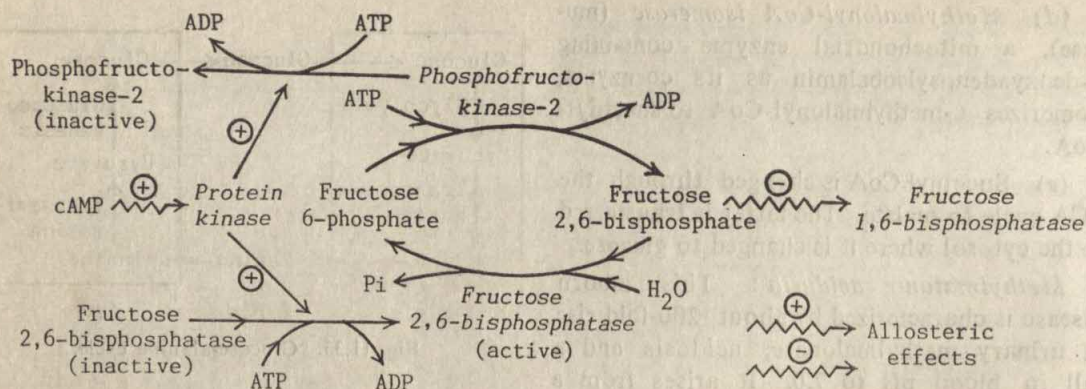


Fig. 11.36. Regulation of fructose 1,6-bisphosphatase by fructose 2,6-bisphosphate.

the affinity of the enzyme for CO₂ and enables the enzyme to form the carboxybiotin intermediate. Fatty acid oxidation promotes gluconeogenesis from pyruvate because (i) acetyl-CoA from fatty acids serves energy purposes and spares pyruvate for gluconeogenesis, (ii) acetyl-CoA as well as NADH from β -oxidation inhibits pyruvate dehydrogenase to reduce aerobic oxidation of pyruvate, and (iii) acetyl-CoA allosterically activates pyruvate carboxylase. ADP inhibits pyruvate carboxylase allosterically.

Glucagon, adrenaline and glucocorticoids induce the synthesis of pyruvate carboxylase and increase gluconeogenesis. Insulin represses the enzyme and reduces gluconeogenesis.

Phosphoenolpyruvate carboxykinase or PEPCK is induced by glucagon during starvation, leading to increased gluconeogenesis. Glucagon increases the intracellular concentration of cAMP which, in turn, increases the transcription of the PEPCK gene into the mRNA meant for translating PEPCK. Insulin reduces gluconeogenesis because its physiological concentrations (10^{-9} to 10^{-6} mM) repress PEPCK by lowering the transcription rate of its gene.

Fructose 1,6-bisphosphatase is strongly and allosterically inhibited by AMP, but is

activated by citrate. So, gluconeogenesis is promoted due to its activation when liver cells are rich in ATP and in fuel molecules such as citrate. Gluconeogenesis is reduced through the inhibition of this enzyme when liver cells are poor in citrate and rich in AMP.

Fructose 2,6-bisphosphate, formed by the phosphorylation of fructose 6-phosphate by phosphofructokinase-2, is a strong allosteric inhibitor of fructose 1,6-bisphosphatase (Fig. 11.36). The intracellular cAMP, increased by glucagon, activates a protein kinase; the latter phosphorylates and inactivates phosphofructokinase-2 to decrease the formation of fructose 2,6-bisphosphate, and simultaneously phosphorylates and activates fructose 2,6-bisphosphatase which hydrolyzes fructose 2,6-bisphosphate. So, glucagon enhances gluconeogenesis by reducing the cellular fructose 2,6-bisphosphate. But insulin dephosphorylates and inactivates fructose 2,6-bisphosphatase and thereby increases the cellular concentration of fructose 2,6-bisphosphate; the latter allosterically inhibits fructose 1,6-bisphosphatase to reduce gluconeogenesis.

Glucagon, adrenaline and glucocorticoids also induce the synthesis of fructose 1,6-bisphosphatase to enhance gluconeogenesis.

Glucose 6-phosphatase is induced by glucagon and cortisol, secreted during starvation. This enhances gluconeogenesis. Insulin represses the enzyme.

High-carbohydrate diets reduce gluconeogenesis by raising the insulin/glucagon ratio and thereby reducing the activities of all four key gluconeogenic enzymes.

11.8 FRUCTOSE METABOLISM

Fructose is an important dietary sugar, largely derived from the intestinal hydrolysis of sucrose.

1. *Fructose 1-phosphate pathway*: (a) The hepatic enzyme *fructokinase* uses ATP to phosphorylate fructose to fructose 1-phosphate (Fig. 11.37). This enzyme occurs also in the intestinal mucosa and kidneys, is specific for fructose and has a much higher fructose-affinity than hexokinases. (b) *Aldolase B* of adult liver cleaves fructose 1-phosphate to dihydroxyacetone phosphate and glyceraldehyde. (c) *Triokinase* of liver uses ATP to phosphorylate glyceraldehyde to glyceraldehyde 3-phosphate. (d) *Aldolase B* may next condense glyceraldehyde 3-phosphate with dihydroxyacetone phosphate to form fructose 1,6-bisphosphate which is converted to glucose in the liver by the steps of gluconeogenesis (page 343). Both glyceraldehyde 3-phosphate and dihydroxyacetone phosphate may also undergo glycolysis. Thus, fructose is either glycolyzed or converted to glucose in the liver.

Human intestinal mucosa changes significant amounts of dietary fructose to glucose by the same pathway. Insulin does not influence fructokinase. This explains the normal utilization of fructose in diabetes.

2. *Fructose 6-phosphate pathway*: In the adipose tissue in particular, some fructose may be phosphorylated to fructose 6-phosphate by *hexokinase*, using ATP as the phosphate donor (Fig. 11.37). Fructose 6-phosphate may then

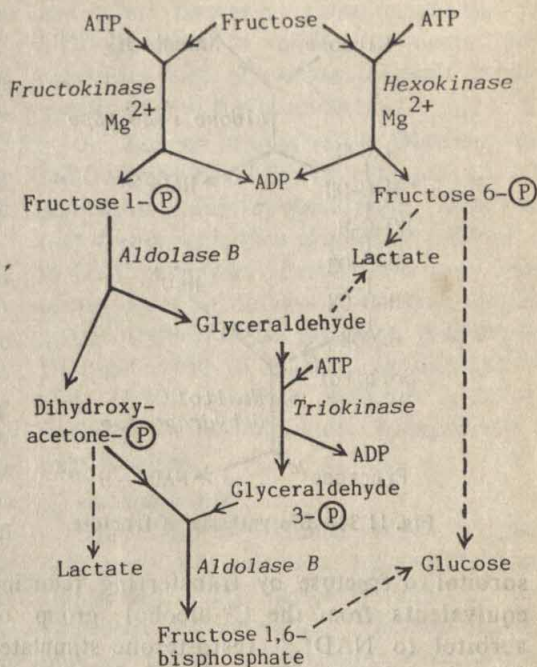


Fig. 11.37. Conversion of fructose to glucose.

be either glycolyzed or converted to glucose. However, hexokinase has a much lower affinity for fructose than for glucose. So, only small amounts of fructose may be normally metabolized in the liver and adipose tissues through this pathway.

3. *Sorbitol pathway for fructose biosynthesis*: Fructose is synthesized from glucose in seminal vesicles of sexually mature males, and secreted in the seminal plasma. It is also synthesized in the placenta of cetaceans and ruminants, and enters their fetal blood and amniotic fluid. Sorbitol, an intermediate in fructose biosynthesis, is also found in the fetal blood of some ruminants.

In the seminal vesicle and ruminant placenta, (a) *aldose reductase* transfers reducing equivalents (H^+ and electrons) from NADPH to glucose, reducing the latter to sorbitol (Fig. 11.38). (b) *Hexitol dehydrogenase* (sorbitol dehydrogenase) then oxidizes

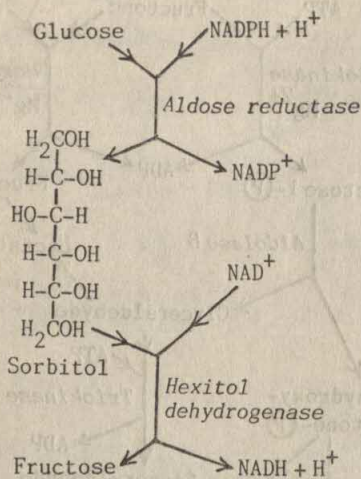


Fig. 11.38. Biosynthesis of fructose.

sorbitol to fructose by transferring reducing equivalents from the C²-alcohol group of sorbitol to NAD⁺. Testosterone stimulates fructose biosynthesis in the seminal vesicle by increasing the activities of both the enzymes. Castration depresses both enzymes.

Genetic errors :

1. *Essential fructosuria*: Fructose is excreted in the urine in this rare genetic disorder due to an inherited deficiency of fructokinase in the liver and a consequent failure to metabolize fructose.

2. *Hereditary fructose intolerance*: In this genetic disorder, fructose 1-phosphate rises in tissues and fructose appears in the urine. This results from a failure to cleave fructose 1-phosphate due to an inherited inability to synthesize aldolase B in the liver. On feeding fructose to the patient, fructose 1-phosphate rises in the blood and allosterically inhibits the liver glycogen phosphorylase to reduce glycogenolysis, leading to hypoglycemia.

3. *Fructose 1,6-bisphosphatase deficiency*: A genetic deficiency of fructose 1,6-bisphosphatase blocks the conversion of fructose 1,6-bisphosphate to glucose and increases the

blood and tissue levels of fructose 1,6-bisphosphate. Hypoglycemia is produced on feeding fructose, because the elevated fructose 1,6-bisphosphate level allosterically inhibits the liver glycogen phosphorylase to reduce glycogenolysis.

11.9 GALACTOSE METABOLISM

Galactose is an important dietary sugar, particularly in infants and children. Intestinal digestion of lactose yields galactose. The latter may also be synthesized from glucose in the body.

1. *Galactose-glucose interconversion pathway*: Liver operates this pathway for converting dietary galactose to glucose (Fig. 11.39).

(a) *Galactokinase* phosphorylates galactose to galactose 1-phosphate with the help of

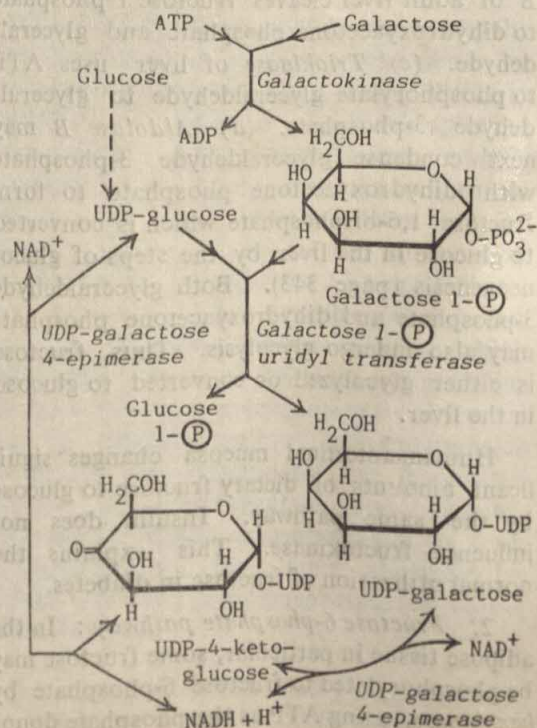


Fig. 11.39. Galactose-glucose interconversion.

ATP and Mg^{2+} . Galactose enhances galactokinase activity.

(b) *Galactose 1-phosphate uridyl transferase* next catalyzes a reaction between galactose 1-phosphate and UDP-glucose to produce UDP-galactose and glucose 1-phosphate. The enzyme causes an interchange of galactose and glucose residues between the reactants.

(c) *UDP-galactose 4-epimerase* isomerizes UDP-galactose to UDP-glucose, using NAD^+ as the coenzyme. First, the epimerase probably oxidizes UDP-galactose to UDP-4-ketoglucose by transferring reducing equivalents (H^+ and electrons) to NAD^+ from the C^4 -alcohol group of the UDP-bound galactose. The NADH thus produced donates the reducing equivalents to the C^4 -ketonyl group of the UDP-bound ketoglucose, reversing the configurations of H and OH groups on its C^4 . This changes UDP-4-ketoglucose to UDP-glucose which may be subsequently used in synthesizing glycogen.

Galactose can also be synthesized from glucose in the liver and lactating mammary glands, using this pathway. Glucose is first changed to UDP-glucose (Fig. 11.2). UDP-galactose 4-epimerase then isomerizes UDP-glucose to UDP-galactose.

2. *Synthesis of glycolipids and glycoproteins*: Because of its high-energy phosphoryl-glycoside bond, UDP-galactose holds galactose in a highly reactive state. So, it can serve as a donor of galactose for the synthesis of glycolipids and glycoproteins. For such synthesis, *galactosyl transferases* of microsomal and Golgi membranes of liver, brain

and other tissues can transfer galactose from UDP-galactose to specific recipients such as ceramides and the oligosaccharide groups of gangliosides and glycoproteins.

3. *Lactose biosynthesis*: Glucose, collected from the blood, is first changed to UDP-glucose in mammary gland cells. *UDP-galactose 4-epimerase* then isomerizes UDP-glucose to UDP-galactose. Finally, *lactose synthase* of the Golgi membranes of lactating mammary glands transfers the galactose residue from UDP-galactose to glucose, producing lactose (Fig. 11.40). Lactose synthase is the *rate-limiting enzyme* of lactose biosynthesis (see page 95 also).

Galactosemia:

This disease results from a genetic deficiency of galactose 1-phosphate uridyl transferase. The defect is inherited as an autosomal recessive trait. The inability of the afflicted infant to metabolize galactose raises the blood galactose level, causes urinary elimination of galactose (*galactosuria*), and increases the galactose 1-phosphate concentration in erythrocytes, brain and liver. These result in symptoms like enlargement and failure of the liver, jaundice, vomiting, diarrhoea and mental retardation. Galactitol is produced in the lens by the reduction of galactose by aldose reductase and causes cataract of the lens. Symptoms may be ameliorated by excluding galactose and lactose from the diet.

11.10 URONIC ACID PATHWAY

This is the metabolic pathway for the synthesis and catabolism of hexuronic acids

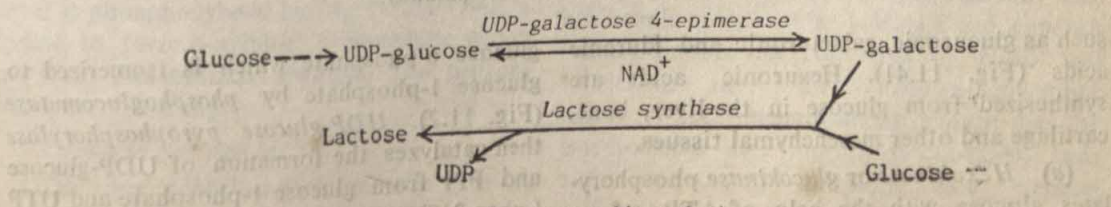


Fig. 11.40. Lactose biosynthesis.

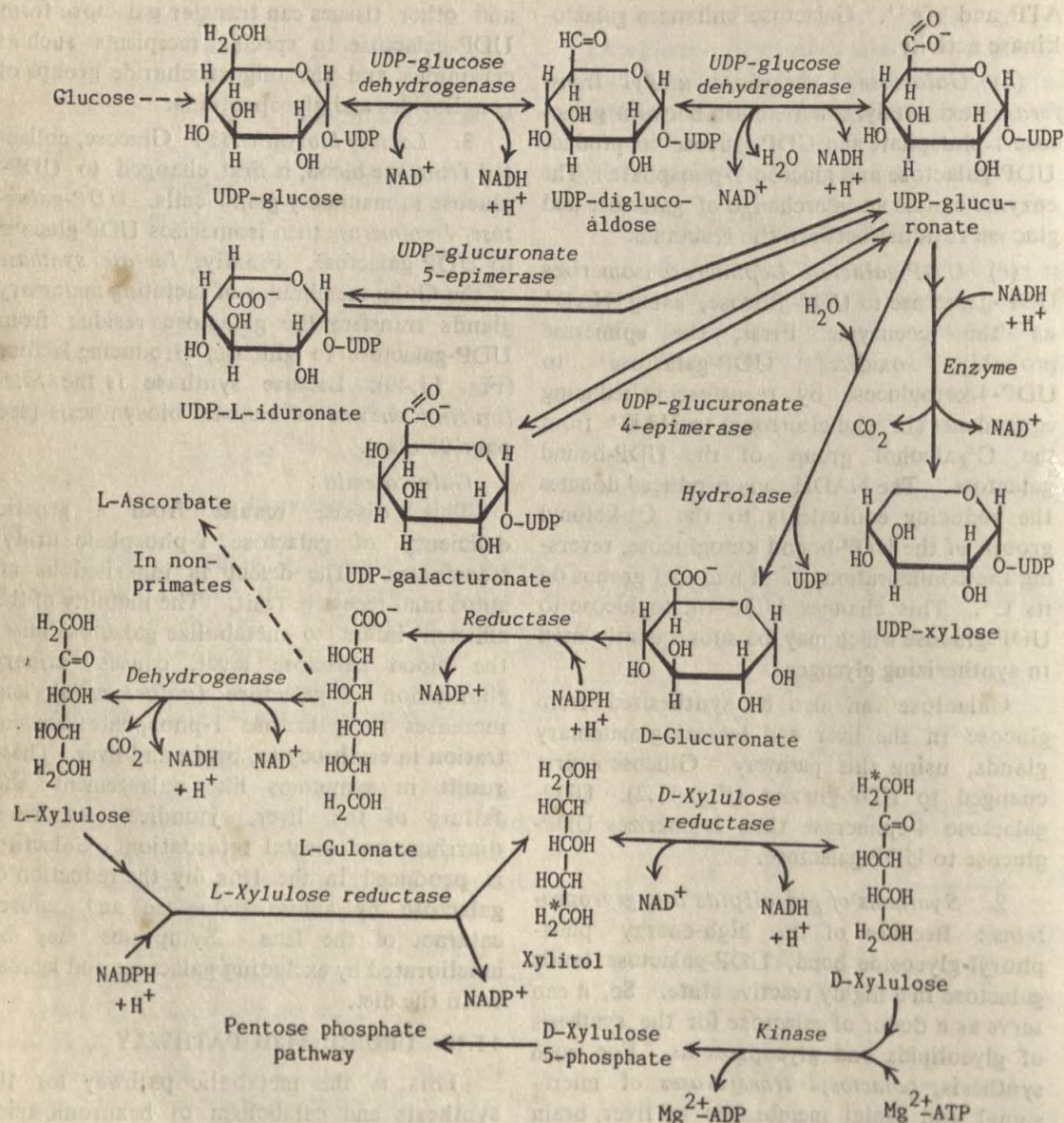


Fig. 11.41. Uronic acid pathway.

such as glucuronic, galacturonic and iduronic acids (Fig. 11.41). Hexuronic acids are synthesized from glucose in the liver, bone, cartilage and other mesenchymal tissues.

(a) *Hexokinase* or *glucokinase* phosphorylates glucose with the help of ATP to form

glucose 6-phosphate which is isomerized to glucose 1-phosphate by *phosphoglucomutase* (Fig. 11.2). *UDP-glucose pyrophosphorylase* then catalyzes the formation of UDP-glucose and PPi from glucose 1-phosphate and UTP (page 310).

(b) *UDP-glucose dehydrogenase* oxidizes UDP-glucose to probably UDP-diglucoaldose, using NAD^+ as the electron-acceptor. The same enzyme then utilizes another NAD^+ and a molecule of water to oxidize UDP-diglucoaldose to UDP-glucuronic acid.

Because of the high-energy phosphoryl-glucoside linkage between UDP and glucuronic acid, *glucuronyl transferases* of smooth endoplasmic reticulum membranes can transfer the glucuronyl group from UDP-glucuronate to growing mucopolysaccharide chains of chondroitin sulfates, heparin and hyaluronic acid in the liver, cartilages and bones, or to steroids, bilirubin, chloramphenicol, etc., during their detoxication in the liver.

(c) *UDP-glucuronate 5-epimerase* and *UDP-glucuronate 4-epimerase* isomerize UDP-glucuronate respectively to UDP-L-iduronic acid and UDP-galacturonic acid. The uronyl groups of these products are transferred to growing mucopolysaccharide chains such as those of dermatan and heparan sulfates.

(d) UDP-glucuronate is decarboxylated and reduced by an NADH-dependent enzyme into UDP-xylose whose xylose group is used for incorporation into glycoproteins.

(e) A *hydrolase* may hydrolyze UDP-glucuronate to liberate UDP and free D-glucuronate. The latter is reduced to L-gulonate by an NADH-dependent reductase. Primate liver oxidizes L-gulonate with the help of an NAD^+ -dependent dehydrogenase to form 3-keto-L-gulonate and decarboxylates the latter to L-xylulose. L-Xylulose is next reduced by NADPH and *L-xylulose reductase* into xylitol which is oxidized to D-xylulose by NAD^+ and *D-xylulose reductase*. The latter is phosphorylated by Mg^{2+} -ATP and a *kinase* to form D-xylulose 5-phosphate which is then metabolized through the pentose phosphate pathway.

Essential pentosuria :

This rare genetic disorder results from an inherited deficiency of the NADPH-dependent

L-xylulose reductase needed for reducing L-xylulose to xylitol. Large amounts of L-xylulose are eliminated in the urine because of a failure to convert it to D-xylulose through xylitol for its normal metabolism in the pentose phosphate pathway.

11.11 HEXOSAMINE METABOLISM

Liver, skin, bone, cartilage, tendon and other mesenchymal tissues synthesize hexosamines from glucose and incorporate them into glycoproteins and gangliosides.

(a) *L-Glutamine D-fructose 6-phosphate amidotransferase* transfers the amide group of glutamine to the C^2 of fructose 6-phosphate formed from glucose. Glucosamine 6-phosphate is thus produced (Fig. 11.42).

(b) *Phosphoglucomutase* isomerizes glucosamine 6-phosphate to glucosamine 1-phosphate which is converted to UDP-glucosamine with the help of UTP and *UDP-glucosamine pyrophosphorylase*. UDP-glucosamine donates its glucosamine group for incorporation into mucopolysaccharides like heparin.

(c) Alternatively, *glucosamine 6-phosphate acetylase* transfers an acetyl group from acetyl-CoA to the C^2 -amino group of glucosamine 6-phosphate, changing the latter to N-acetylglucosamine 6-phosphate.

(d) N-Acetylglucosamine 6-phosphate is isomerized by a *mutase* to N-acetylglucosamine 1-phosphate which is converted to UDP-N-acetylglucosamine by reacting with UTP with the help of *UDP-glucosamine pyrophosphorylase*.

(e) UDP-N-acetylglucosamine may either donate its N-acetylglucosamine group for incorporation into mucopolysaccharides like hyaluronic acid, keratan sulfate I and heparan sulfate, or become converted by NAD^+ and *4-epimerase* into UDP-N-acetylgalactosamine whose N-acetylgalactosamine group may be incorporated into mucopolysaccharides such as chondroitin, dermatan and keratan sulfates.

(f) N-Acetylglucosamine 6-phosphate may also be epimerized by a 2-epimerase into N-acetylmannosamine 6-phosphate. *Enolpyruvyl transferase* (N-acetylneuraminate 9-phosphate synthase) transfers the enolpyruvate group from phosphoenolpyruvate to N-acetylmannosamine 6-phosphate to produce P_i and N-acetylneuraminate 9-phosphate. The latter is hydrolyzed by a phosphatase into N-acetylneuraminate or *sialic acid*, a C_9 amino sugar, which is converted to CMP-sialic acid by reacting with CTP (cytidine triphosphate) under the action of *acylneuraminate cytidyl transferase* (Fig. 11.42). The sialyl group of CMP-sialic acid is incorporated into mucopolysaccharides and gangliosides.

The rate of amino sugar biosynthesis is largely regulated by controlling L-glutamine D-fructose 6-phosphate amidotransferase which

catalyzes the *rate-limiting step* of the pathway. This enzyme is allosterically inhibited by the feed-back effect of N-acetylglucosamine 6-phosphate.

11.12 BLOOD SUGAR

Glucose is the principal sugar of mammalian blood. It normally amounts to $65-110 \text{ mg dl}^{-1}$ twelve hours after a meal (*fasting blood sugar*). The sugar concentration is similar in plasma and erythrocytes, but is usually 2-8 mg lower in venous blood than in arterial blood.

Ruminants possess lower blood sugar levels; the fasting blood sugar usually amounts to $40-60 \text{ mg dl}^{-1}$ in cow. Ruminant fetal blood contains fructose also. Insect hemolymph contains trehalose as the principal sugar.

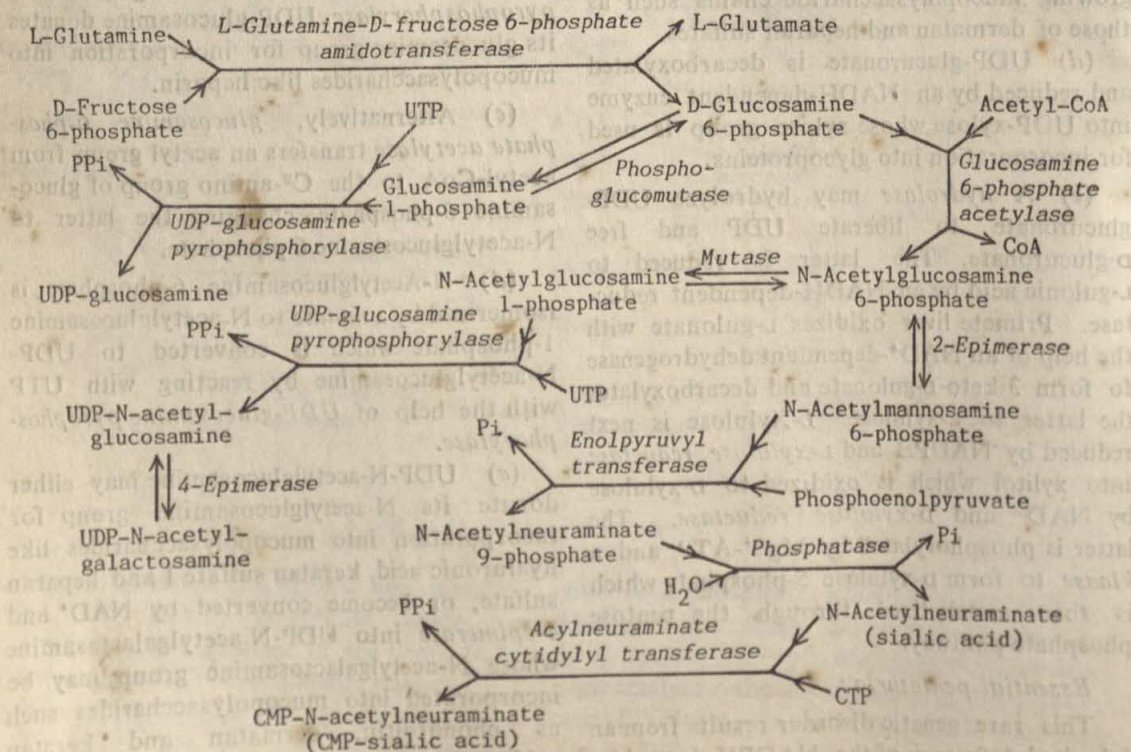


Fig. 11.42. Amino sugar pathway.

Hyperglycemia is a rise in the blood sugar (e.g., in diabetes mellitus) while *hypoglycemia* is a fall in it (e.g., in starvation and hyperinsulinism).

Sources

(a) Glucose absorbed from the intestine is the major source of blood sugar. Human blood glucose rises normally upto 160 mg dl^{-1} after a high-carbohydrate meal.

(b) Liver adds glucose to blood by *glycogenolysis* of stored glycogen.

(c) Liver and kidneys add glucose to blood by *gluconeogenesis* from amino acids, propionate, lactate and glycerol.

Fates

(a) Storage as glycogen in liver, muscles and many other tissues.

(b) Catabolism for energy production in all tissues, particularly muscles.

(c) Conversion to fat for storage in adipose tissues.

(d) Conversion to amino acids, fructose, lactose, pentoses, hexosamines, hexuronic acids, glycerol, glycolipids, etc., in liver, brain, mammary glands, seminal vesicles and other tissues.

Functions

Blood sugar is mainly intended for the continuous supply of glucose to all tissues including muscles, brain and blood cells, for energy production. It is also utilized by tissues in synthesizing amino acids, glycerol, fats, glycolipids, pentoses, lactose and other substances.

Regulation

This is discussed in the chapter on Hormones.

11.13 GLUCOSE TOLERANCE

It is the capacity of the body to store and metabolize glucose. It is estimated from

changes in the blood glucose level after glucose administration. A reduced tolerance (e.g., in diabetes mellitus) causes an abnormally high and prolonged hyperglycemia after glucose administration. As long as the blood sugar does not exceed $170\text{--}180 \text{ mg dl}^{-1}$, all the sugar filtered into renal tubules may be absorbed by tubule cells, leaving almost no sugar in the urine. This level of blood sugar, called the *renal threshold for glucose*, may be exceeded in case of reduced sugar tolerance so that the kidneys fail to reabsorb all the filtered glucose and glucose appears in the urine (*glucosuria*). A heightened sugar tolerance causes hypoglycemia (e.g., in hyperinsulinism and Addison's disease).

Oral glucose tolerance test

The patient should take sufficient amounts of carbohydrates for several days preceding the test because a low-carbohydrate diet may temporarily reduce the tolerance. He should not take anything on the morning of the test to retain the fasting state. A venous blood sample is first collected from the patient. He then drinks a solution of 75 g of glucose in 250 ml of water. Venous blood samples are then collected every 30 minutes upto 3 hrs after the glucose intake. Glucose is estimated in all the blood samples and the results are plotted against time intervals to get the tolerance curve (Fig. 11.43).

Normally, the fasting blood sugar does not equal or exceed 120 mg dl^{-1} ; also, the blood sugar reaches a peak nearing even $160\text{--}170 \text{ mg dl}^{-1}$ in an hour after feeding, does not exceed the renal threshold for glucose and falls within 2 hours of intake to the fasting level or lower. In diabetes, the fasting blood sugar equals or exceeds 120 mg dl^{-1} ; also, the blood sugar reaches a peak higher than the renal threshold in $2\text{--}2\frac{1}{2}$ hours and takes more than $2\frac{1}{2}$ hours to fall to the fasting level. In case of increased tolerance, the fasting blood sugar is below

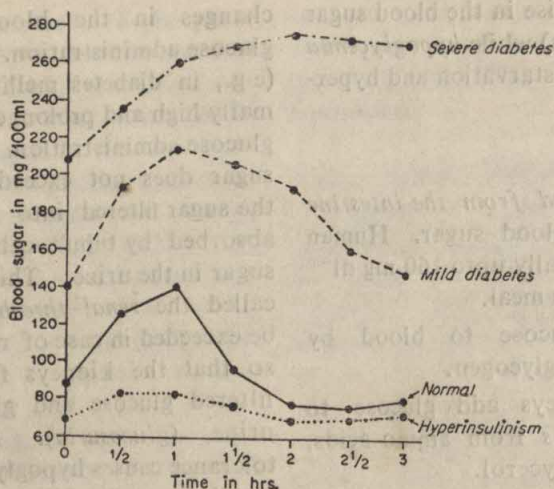


Fig. 11.43. Oral glucose tolerance curves.

60 mg; also, the blood sugar rises only by 10-30 mg after glucose intake, follows a somewhat flat course and finally shows a pronounced hypoglycemia.

Intravenous glucose tolerance test

This is undertaken when either the intestinal absorption is impaired or the oral administration is not possible. A sample of venous blood is collected from the patient. He is then intravenously injected with a 20% solution of 25 g glucose and venous blood samples are collected every 30 minutes upto 2 hours. Blood glucose is then estimated in all the samples and the tolerance curve is plotted. Normally, the blood sugar would take about $\frac{1}{2}$ hour to reach a peak lower than 250 mg dl⁻¹ and would decline to the fasting level or lower in 1-1 $\frac{1}{2}$ hours after injection.

Postprandial sugar estimation

The sugar is estimated in a blood sample collected from the patient exactly 2 hours after either a normal carbohydrate-containing meal or an intake of 75 g glucose dissolved in 250 ml water. The postprandial sugar, so determined, should not normally exceed 120 mg dl⁻¹.

11.14 GLYCOSYLATED HEMOGLOBIN

Glucose entering the erythrocytes may bind with hemoglobin A (HbA) molecules to form glycosylated hemoglobins such as HbA₁ and HbA_{1c}. The amount of these glycohemoglobins depends upon the blood glucose level and rises with the rise in blood glucose. At any time, the average age (i.e., half the total life span) of all circulating erythrocytes amounts to 2 months. So, the amount of glycohemoglobins serves as an estimate of the average blood sugar level over the preceding 2 months. The glycohemoglobin value is usually expressed as the amount of glycohemoglobin (HbA₁ or HbA_{1c}) as the percentage of total hemoglobin. A glycohemoglobin (HbA₁) value of 5.4-7.4% is obtained in nondiabetic individual with an average blood glucose of less than 120 mg dl⁻¹ over the last 2 months. A value of 10.6-13.0% indicates an average blood glucose of 200-300 mg dl⁻¹ in a diabetic. Although glycohemoglobin value is useful in the assessment and management of diabetes, it gives no indication about the variations in blood glucose in a single day.

12. METABOLISM OF PROTEINS

Food proteins are mainly absorbed as amino acids. Some amino acids are also synthesized in the body. Amino acids are used in the synthesis of structural proteins of tissues, catalytic proteins (enzymes), carrier proteins, receptor proteins, antibody proteins, many informational molecules (e.g., peptide hormones, catecholamines, serotonin, GABA, iodothyronines), some pigments (e.g., porphyrins, melanins), creatine, purines, pyrimidines, polyamines, nicotinamide and carbohydrates. When necessary, amino acids are catabolized for energy production.

12.1 NON-PROTEIN NITROGEN

Nitrogen is present in foods, tissues and body fluids, mostly as proteins. But nitrogen also occurs in body fluids and urine as non-protein nitrogenous substances (NPN). These include nitrogenous catabolites such as urea, ammonia, uric acid and creatinine, amines

like histamine and catecholamines, purines, pyrimidines, creatine and free amino acids. Urinary nitrogen normally consists almost solely of NPN.

12.2 NITROGEN BALANCE

When the total amount of dietary nitrogen, absorbed from the alimentary canal, equals the total amount of nitrogen eliminated from the body in urine, sweat and gastrointestinal secretions, the body is in *nitrogen equilibrium* with no change in its amount of nitrogen. In this state, the rates of protein synthesis and catabolism are identical in the body. Ordinarily, normal healthy adults are in nitrogen equilibrium.

Negative nitrogen balance is a state where the nitrogen output exceeds its intake and there is a consequent loss of nitrogen from the body. In other words, the rate of protein catabolism exceeds that of protein synthesis, resulting in a decline in tissue proteins. Wasting diseases, surgical or medical trauma, old age, protein malnutrition (e.g., kwashiorkor and marasmus), starvation, and deficiency of essential amino acids may cause a negative N-balance due to a continuing loss of tissue proteins.

Whenever some of the dietary nitrogen is retained in the body as tissue protein, the intake of nitrogen exceeds its output from the body and *positive nitrogen balance* ensues. There is a rise in tissue protein content because the rate of protein synthesis exceeds that of protein catabolism. This happens during growth, pregnancy and convalescence.

TABLE 12.1. NPN in normal human blood and urine.

NPN	In blood (mg dl ⁻¹)	In urine (g day ⁻¹)
Total NPN	20—35	8—14
Amino acid N	3—6*	0.1—0.2
Ammonia	<0.11	0.3—1.2
Creatine	0.2—0.6*	<6
Creatinine	0.7—1.2*	1.2—1.5
Hippurate	—	0.5—0.9
Urea N	8—25*	6—13
Uric acid	3—9*	0.2—0.8

* mg per dl serum.

12.3 METABOLIC CLASSIFICATION OF AMINO ACIDS

From the *nutritional standpoint*, amino acids belong to two classes.

1. *Essential amino acids* :

The carbon skeletons of some amino acids cannot be synthesized in the body. So, these amino acids must be supplied in the diet and are called essential or indispensable amino acids. Body cannot store free amino acids and these are promptly catabolized unless immediately built up into proteins; so, deficiency of an essential amino acid not only causes a failure of protein synthesis but also increases the catabolic loss of all other available amino acids due to a failure of their utilization. This results in negative N-balance, growth failure and fall in plasma proteins and hemoglobin; nausea, dizziness, muscular incoordination, increased urinary excretion of non-keto acids and a fall in sperm count may also occur. Following eight essential amino acids are required for maintaining N-equilibrium in man; their requirements in g per kg of dietary proteins are given within parentheses; methionine (22); threonine (28); tryptophan (14); valine (42); isoleucine (42); leucine (48); phenylalanine (28); lysine (42).

In addition to those eight essential amino acids, histidine and arginine are considered *semi-indispensable amino acids*. Both amino acids are essential in the infant diet because the rates of histidine biosynthesis and of the urea synthesis cycle producing arginine as an intermediate, are inadequate for providing those amino acids in amounts required for growth. Even in adult man, dietary arginine deficiency reduces the sperm count, though not causing negative N-balance. Adult patients on prolonged parenteral nutrition, deficient in histidine, suffer from negative N-balance and a scaly erythematous dermatitis which improves on histidine administration.

Animal proteins of high biological values (e.g., milk, liver and egg proteins) contain all essential amino acids in adequate amounts. But the maize protein zein is poor in tryptophan and lysine, sesame protein is deficient in lysine and valine, gliadin of wheat is poor in lysine, legumes are poor in methionine and tryptophan, while sorghum protein is deficient in tryptophan.

Oser proposed the *essential amino acid index* as a measure of the nutritional value of a protein in terms of its essential amino acid content. This index is the geometric mean of the ratios of the amounts of all the essential amino acids present in the protein to their respective amounts required by an animal for maintaining N-equilibrium and growth.

2. *Non-essential amino acids* :

Amino acids like glycine, serine, tyrosine, cysteine, alanine, aspartate, glutamate, proline and hydroxyproline are non-essential in human food, because their carbon skeletons can be synthesized and transaminated in the body to form these amino acids in adequate amounts. However, requirements for specific non-essential amino acids may be manifold enhanced under some conditions, making them essential in the diet. For example, prolonged excessive intakes of benzoic acid use up so much glycine for getting detoxicated that a negative N-balance results unless adequate amounts of glycine are provided in the diet.

According to their *metabolic fates*, amino acids belong to three classes :

1. *Glycogenic amino acids* :

They include glycine, alanine, serine, threonine, cysteine, valine, methionine, glutamate, aspartate, histidine, arginine, proline and hydroxyproline. After removal of the amino group, their carbon skeletons form gluconeogenic intermediates such as propionate, pyruvate, oxaloacetate and α -keto-glutarate. These amino acids raise blood

sugar and liver glycogen, when fed to starving animals, and increase urinary sugar in diabetic or phlorrhizinized animals.

2. Ketogenic amino acids :

Carbon skeletons of leucine and lysine yield acetoacetate to increase ketosis and ketonuria in diabetic animals.

3. Glycogenic-ketogenic amino acids :

Their carbon skeletons change partly to gluconeogenic products and partly to ketone bodies. Phenylalanine and tyrosine form acetoacetate and fumarate. Tryptophan produces acetoacetate and pyruvate. Isoleucine changes into propionyl-CoA and acetyl-CoA.

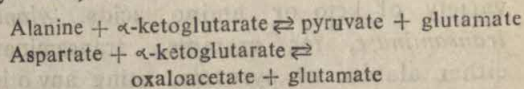
12.4 METABOLISM OF AMINO ACIDS

Nitrogen catabolism consists of the removal of α -amino groups of amino acids as ammonia by transamination and deamination, and the conversion of the released ammonia to excretory endproducts such as urea and uric acid. *Catabolism of carbon skeletons*, left after nitrogen catabolism, consists of their change to amphibolic intermediates such as gluconeogenic substances and ketone bodies, and their oxidation in the TCA cycle. In addition, amino acids are also converted to specific specialized products like porphyrins, creatine and melanins.

12.5 TRANSAMINATION

Transamination transfers the amino group of an amino acid (e.g., alanine) to a keto acid (e.g., α -ketoglutarate), changing the latter into a new amino acid (e.g., glutamate) and the

original amino acid into a new keto acid (e.g., pyruvate).



Transaminases or *aminotransferases* catalyze transaminations in mitochondria and cytoplasm of liver in particular, and also of kidneys, heart, testes and brain. They carry pyridoxal phosphate (PLP) as the prosthetic group. Mammalian transaminases act specifically on L-amino acids, but not on their D-isomers.

Except threonine, lysine, proline and hydroxyproline, all α -amino acids, glutamine and asparagine can participate in transaminations and change into the respective α -keto acids by donating their α -amino groups. The corresponding α -keto acids, including glyoxylate, can act as amino-acceptors in transaminations and thereby change to the respective α -amino acids. Transaminations are freely reversible and can serve in both the formation of an amino acid from its α -keto acid and the catabolism of the former to the latter (Fig. 12.1).

Each transaminase is specific for one pair of substrates ; i.e., it always uses a specific α -amino acid or the corresponding α -keto acid as one of the substrates. So, it always catalyzes either the removal of the amino group from that specific amino acid or the addition of an amino group to the corresponding keto acid. But it is nonspecific for the other pair of substrates ; so, it can

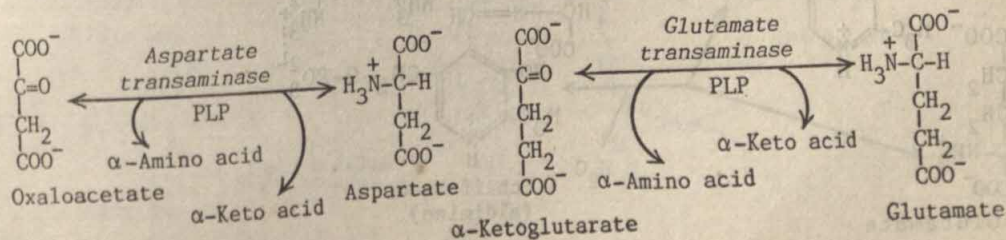


Fig. 12.1. Transaminations of aspartate and glutamate.

transfer the amino group between the specific amino or keto acid and any one of a wide variety of keto or amino acids. *Alanine transaminase*, for example, transaminates either alanine or pyruvate, using any other keto or amino acid respectively. Though present in most tissues, its activity is highest in the liver. Its activity in the serum, usually known as the SGPT activity, rises abnormally in hepatic lesions (page 112). *Aspartate transaminase*, richest in the cardiac muscle,

transaminates either aspartate or oxaloacetate using some other keto or amino acid as the other substrate. Its activity in the serum, usually called the SGOT activity, rises abnormally in myocardial infarctions (page 112). *Glutamate transaminase* acts specifically on glutamate or α -ketoglutarate as one of its substrates, but uses any of a large number of amino acids or their corresponding keto acids as the other substrate. It is widely distributed in tissues and its activity is

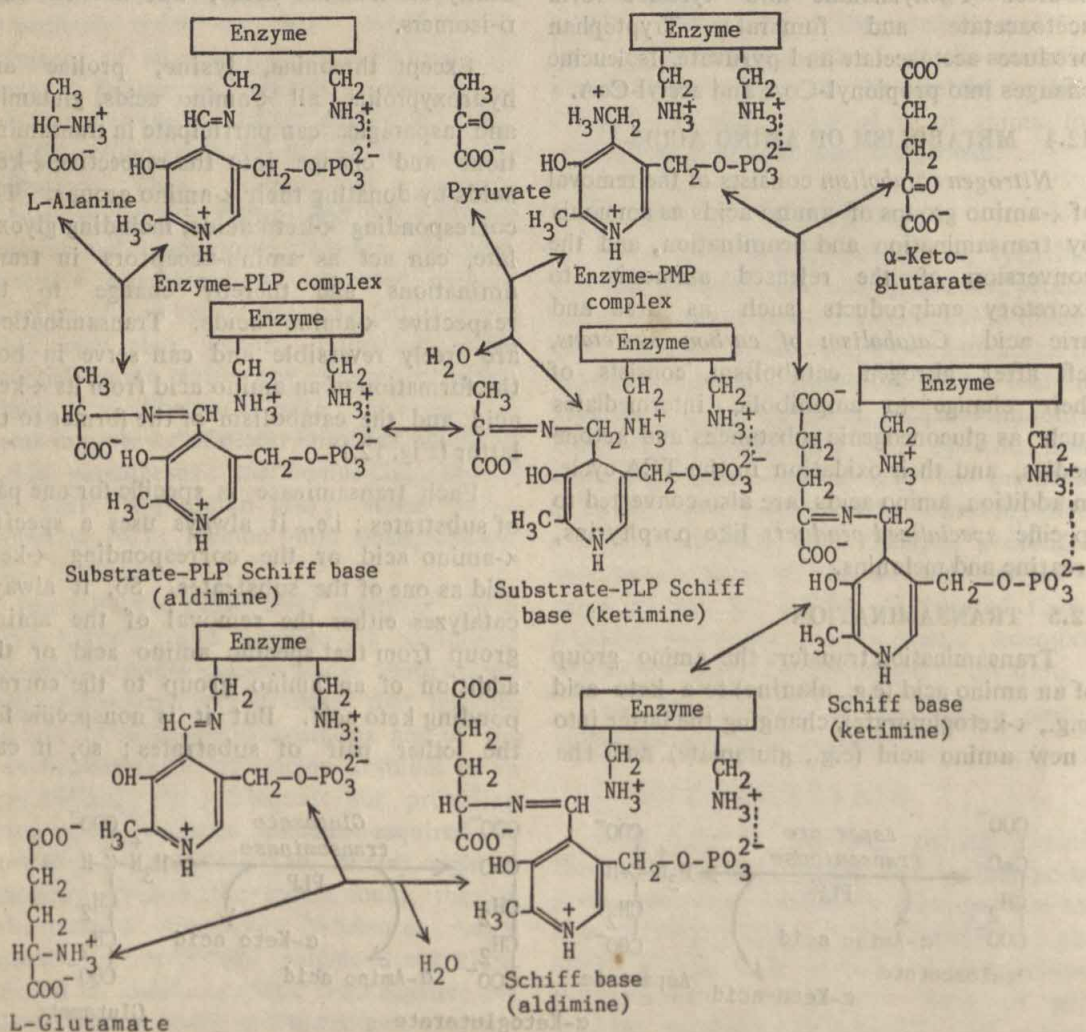


Fig. 12.2. Mode of action of a transaminase. Substrates: alanine and α -ketoglutarate.

enhanced during protein catabolism and gluconeogenesis. Another widely distributed enzyme, *glycine transaminase*, is specific for glycine and glyoxylate.

Transaminations are double-displacement (ping-pong) type of bisubstrate reactions. The two substrates, viz, an amino acid and an α -keto acid, bind separately and successively with the prosthetic group of the enzyme.

(i) In absence of substrates, the PLP prosthetic group remains bound to the apoenzyme by (a) an electrostatic bond between a cationic lysine residue of the apoenzyme and the anionic phosphate group of PLP, and (b) a covalent Schiff base linkage between another active-site lysine residue of the apoenzyme and the aldehyde group of PLP (page 151). The substrate amino acid displaces the active-site lysine residue from the pre-existing Schiff base linkage and in its place, itself forms a new Schiff base *aldimine* with the PLP still held electrostatically by the enzyme (Fig. 12.2). This aldimine changes into a *ketimine* by a shift of the Schiff base double-bond. The ketimine then gets hydrolyzed to release an α -keto acid as a product, leaving pyridoxamine phosphate (PMP) as the enzyme-bound prosthetic group.

(ii) The α -keto acid which is the second substrate, now binds with the PMP to form

a new Schiff base ketimine which changes into an aldimine. The latter is cleaved to release an amino acid as the second product, because the active-site lysine displaces it from its Schiff base and reforms the original Schiff base with the PLP left behind.

The reaction may be summarized as follows :

- $$\begin{aligned} (i) & \alpha\text{-Amino acid}_1 + \text{enzyme-PLP} \rightleftharpoons \alpha\text{-keto acid}_1 + \text{enzyme-PMP} \\ (ii) & \alpha\text{-Keto acid}_2 + \text{enzyme-PMP} \rightleftharpoons \alpha\text{-amino acid}_2 + \text{enzyme-PLP} \end{aligned}$$

Transamination (i) *redistributes amino-N* among amino acids, forming new amino acids with the amino groups of pre-existing ones, (ii) *removes amino groups* from amino acids by transferring these amino groups to α -ketoglutarate, pyruvate or glyoxylate, and (iii) *produces gluconeogenic products* such as pyruvate and oxaloacetate.

12.6 TRANSDEAMINATION

This process is of considerable quantitative importance in the liver for removing amino groups from amino acids. It consists of transamination followed by oxidative deamination.

Transdeamination involving glycine

(a) *Glycine transaminases* first transfer the amino group from either glutamate or

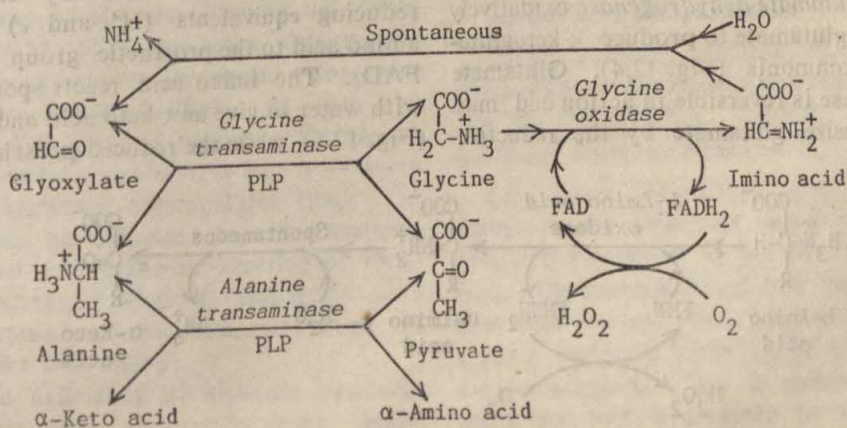


Fig. 12.3. Transdeamination involving glycine.

alanine to glyoxylate, producing glycine and either α -ketoglutarate or pyruvate (Fig. 12.3). These PLP-containing enzymes, though reversible in action, appear to act *in vivo* almost solely in transaminating glyoxylate to glycine. As glutamate and alanine transaminases are widely distributed and transfer amino groups of most other amino acids to α -ketoglutarate and pyruvate respectively, this step ensures the transfer of amino groups of most amino acids to glyoxylate to form glycine,

(b) *Glycine oxidase*, a flavoprotein containing FAD, next catalyzes the oxidative deamination of glycine. The enzyme oxidizes glycine to an imino acid by transferring reducing equivalents (H^+ and e) from glycine to FAD which is thereby reduced to $FADH_2$. The imino acid reacts spontaneously with water to produce glyoxylate and ammonia while $FADH_2$ is reoxidized with molecular O_2 to give FAD and H_2O_2 .

A genetic deficiency of glycine oxidase causes a failure of glycine catabolism to produce *hyperglycinemia* with high blood glycine level and mental retardation.

Transdeamination involving glutamate

(a) *Glutamate transaminase* first transfers the amino group of an amino acid to α -ketoglutarate, producing a new α -keto acid and glutamate.

(b) *L-Glutamate dehydrogenase* oxidatively deaminates glutamate to produce α -ketoglutarate and ammonia (Fig. 12.4). Glutamate dehydrogenase is reversible in action and may also synthesize glutamate by the reductive

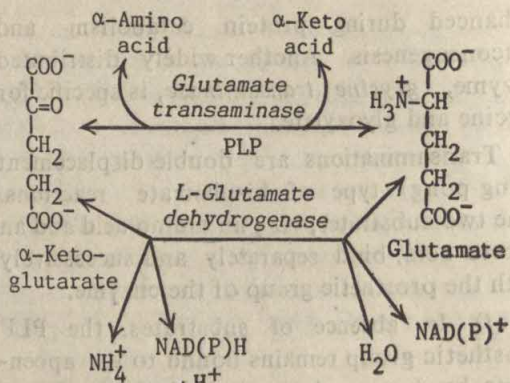


Fig. 12.4. Transdeamination involving glutamate.

amination of α -ketoglutarate with free ammonia. Cytoplasmic glutamate dehydrogenase has a low K_m for ammonia and mainly catalyzes the biosynthesis of glutamate, using NADPH. The mitochondrial enzyme uses NAD^+ and mainly serves to deaminate other amino acids through glutamate and to provide α -ketoglutarate for the TCA cycle. The mitochondrial enzyme is allosterically inhibited by NADH, ATP and GTP; it is allosterically activated by ADP, GDP and AMP.

12.7 OXIDATIVE DEAMINATION BY AMINO ACID OXIDASES

Flavoprotein enzymes called *amino acid oxidases* may oxidize an amino acid to the corresponding imino acid by transferring reducing equivalents (H^+ and e) from the amino acid to the prosthetic group (FMN or FAD). The imino acid reacts spontaneously with water to give an α -keto acid and ammonia (Fig. 12.5) while the reduced prosthetic group

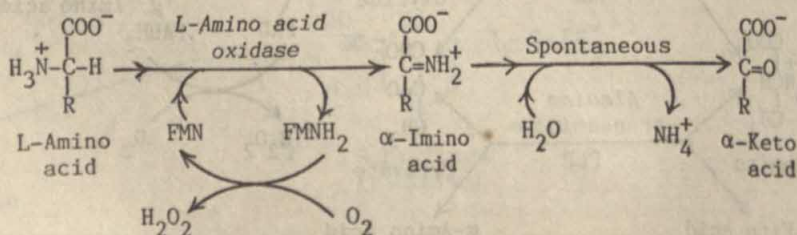


Fig. 12.5. Oxidative deamination by L-amino acid oxidase.

(FMNH₂ or FADH₂) is reoxidized directly by molecular O₂, producing H₂O₂.

L-Amino acid oxidase carries FMN and oxidatively deaminates *L*-amino acids except S-containing, β -hydroxy, dicarboxylic and basic amino acids. It is ineffective on glycine. It occurs in mammalian renal mitochondria and microsomes, but is too poor in the mammalian liver to play any major role. *D*-Amino acid oxidase occurs in mammalian hepatic and renal peroxisomes, carries FAD, and oxidatively deaminates *D*-amino acids either to destroy them or to help them change into their *L*-isomers which are the only natural amino acids in mammals.

12.8 NON-OXIDATIVE DEAMINATION

Several groups of enzymes catalyze the deamination of amino acids, involving no oxidation. This is much less important than transamination and transdeamination.

Amino acid lyases

These *C*-*N* lyases deaminate significant amounts of histidine and aspartic acid.

(a) *Histidase* (histidine ammonia-lyase) occurs in the liver and the cutaneous stratum corneum. It non-oxidatively deaminates histidine, yielding urocanic acid and ammonia (Fig. 12.6). Urocanate is further catabolized by *urocanase* in the liver. Because urocanase is absent in the skin, urocanate accumulates there and is excreted in the sweat. Urocanate reduces the photosensitivity of skin by absorbing UV rays of sunlight. Cutaneous hypersensitivity to sunlight, as seen in pellagra and acrodermatitis enteropathica (page 133), results from an enhanced hepatic urocanase activity and a consequent lowering of cutaneous urocanate. Histidine deficiency also lowers cutaneous urocanate, resulting in sunburn-like skin lesions.

Genetic deficiency of histidase produces *histidinemia* with abnormally high blood histidine level and urinary elimination of large

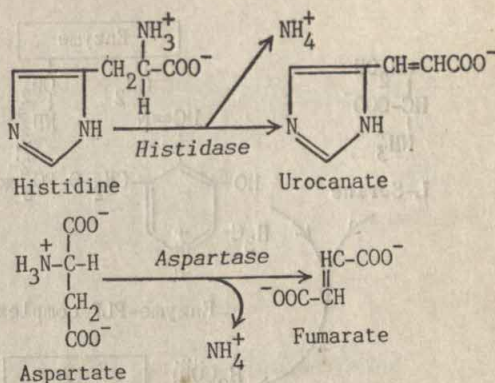


Fig. 12 6. Action of amino acid lyases.

amounts of histidine and its metabolites like imidazole pyruvate, imidazole lactate and imidazole acetate.

Hepatic histidase is induced by glucagon, cortisol and estrogens, but repressed by triiodothyronine (T₃). So, starvation which stimulates cortisol and glucagon secretions, enhances histidase activity leading to histidine catabolism and gluconeogenesis from its metabolic endproduct.

(b) *Aspartase* (aspartate ammonia-lyase) non-oxidatively deaminates aspartate to ammonia and fumarate. In many species, it may also catalyze the reverse reaction, viz., the *reductive amination* of fumarate to aspartate with free ammonia.

Amino acid dehydratases

These enzymes contain pyridoxal phosphate (PLP) as the prosthetic group and catalyze the dehydration, followed by deamination, of hydroxy amino acids.

(a) *Serine dehydratase* (serine deaminase) occurs in the liver and catalyzes the dehydration deamination of both serine and threonine. The enzyme-bound PLP forms a Schiff base with serine (Fig. 12.7). Elimination of a water molecule from serine changes it to α -aminoacrylate. This is released from the Schiff base and hydrolyzed to ammonia and pyruvate.

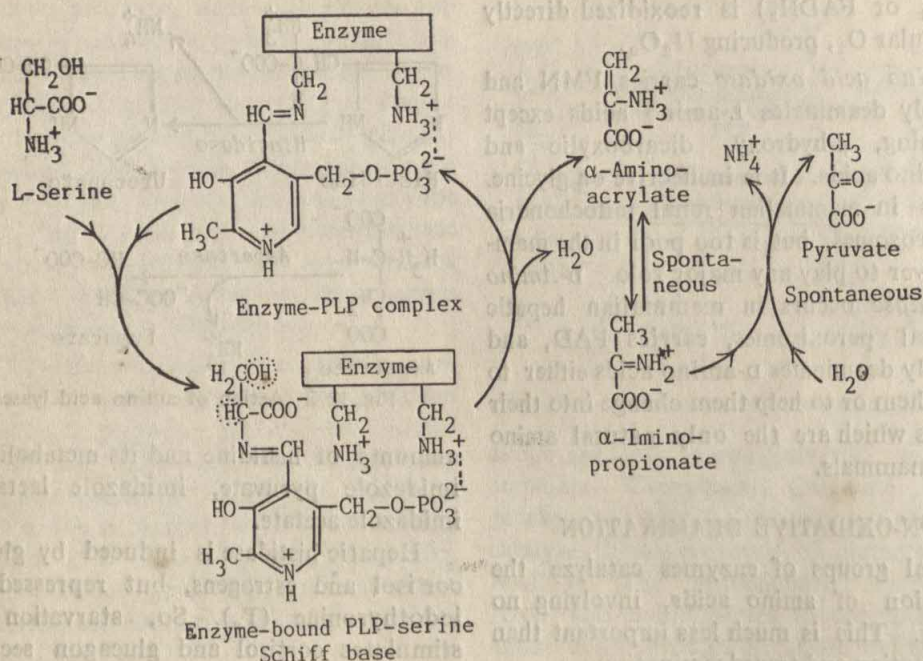


Fig. 12.7. Mode of action of serine dehydratase.

One of the serine dehydratase isoenzymes of rat liver is induced by glucocorticoids and repressed by insulin while its other isoenzyme is induced by glucagon. Starvation increases the secretions of adrenal glucocorticoids

and glucagon, but decreases insulin secretion. Thereby, starvation enhances the activities of both isoenzymes. This increases the deamination of serine to pyruvate for gluconeogenesis.

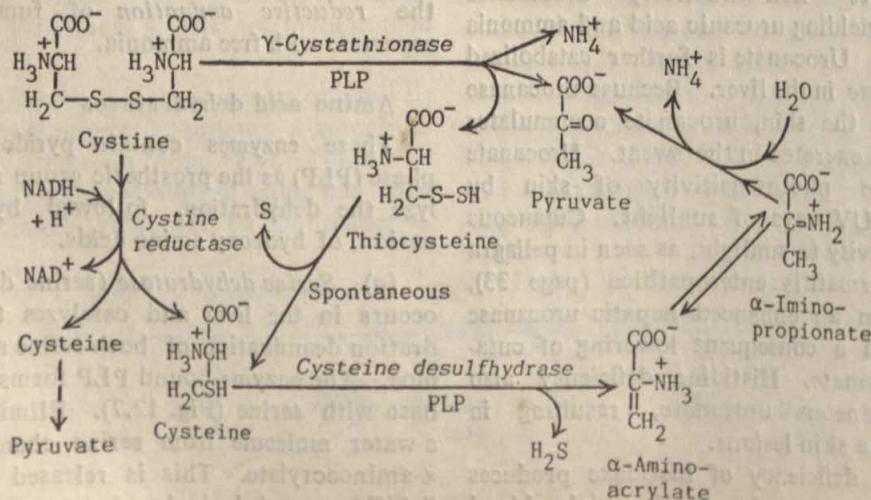


Fig. 12.8. Desulfhydration deamination of cysteine and cystine.

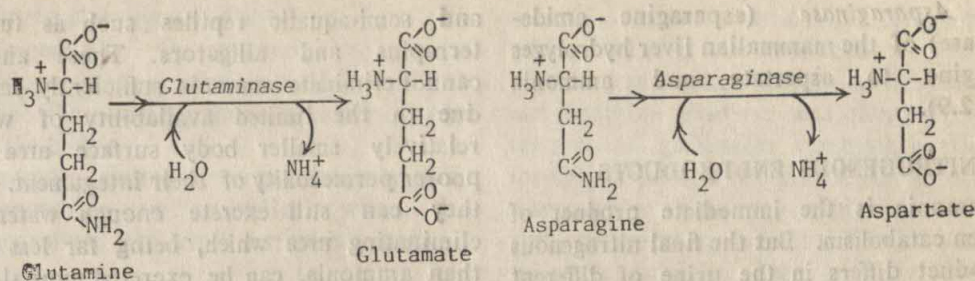


Fig. 12.9. Actions of glutaminase and asparaginase.

High-protein diets also increase gluconeogenesis from serine by reducing the K_m of serine dehydratase for serine.

(b) *Threonine dehydratase* (threonine deaminase) of the mammalian liver catalyzes the dehydration deamination of threonine to ammonia and α -ketobutyrate.

Amino acid desulfhydrases

(a) *Cysteine desulfhydrase* of the mammalian liver is a PLP-containing enzyme which catalyzes desulfhydration and then deamination of cysteine. Its mode of action resembles that of serine dehydratase on serine (Fig. 12.8). Cysteine is converted to H₂S, NH₄⁺ and pyruvate through α -aminoacrylate.

(b) γ -*Cystathionase* is another PLP-containing enzyme of the mammalian liver. It may act as a cystine desulfhydrase to catalyze the deamination and desulfhydration of cystine into pyruvate, ammonia, cysteine and elemental sulfur (Fig. 12.8).

Amino acid amide hydrolases

These enzymes catalyze hydrolytic deamination of glutamine and asparagine, releasing their amide groups as ammonia and changing them respectively to glutamate and aspartate.

(a) *Glutaminase* (glutamine amide-hydrolase) hydrolyzes glutamine practically irreversibly to ammonia and glutamate (Fig. 12.9). Different glutaminase isoenzymes occur in the inner mitochondrial membrane of liver and kidneys and the brush-border of proximal renal tubules. Glutaminase II, one of the hepatic glutaminases, consists of a *glutamine transaminase* for transaminating glutamine to α -ketoglutarate, and an ω -*amidase* for hydrolyzing the latter into ammonia and α -ketoglutarate (Fig. 12.10). Unlike hepatic isoenzymes, renal glutaminase activity increases considerably during acidosis and is particularly high in ammonotelic animals where it accounts for the urinary elimination of ammonia as their chief nitrogenous endproduct.

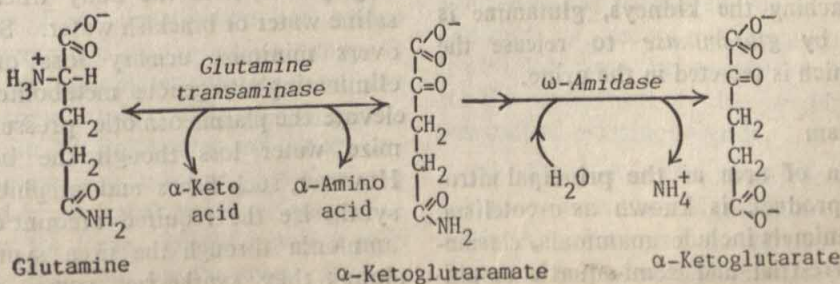


Fig. 12.10. Enzyme activities of glutaminase II.

(b) *Asparaginase* (asparagine amide-hydrolase) of the mammalian liver hydrolyzes asparagine to aspartate and ammonia (Fig. 12.9).

12.9 NITROGENOUS ENDPRODUCTS

Ammonia is the immediate product of nitrogen catabolism. But the final nitrogenous endproduct differs in the urine of different animal species, depending largely on the availability of water for its efficient elimination in the natural habitat. Most commonly it is ammonia, urea or a purine like uric acid.

Ammonotelism

Elimination of nitrogenous endproduct mostly or wholly as ammonia, is known as ammonotelism. Ammonia is highly toxic and must be speedily removed from the body. It is highly soluble and freely diffusible. So, its elimination poses no problem in small animals with large body surface area for rapid outward diffusion of ammonia, or in aquatic animals. Earthworms and many aquatic invertebrates, teleost fishes (e.g., Rohu, Pomfret), aquatic amphibian larvae like tadpoles, adult aquatic amphibians (e.g., *Xenopus*, *Pipa*, salamanders) and even some aquatic turtles are primarily ammonotelic. Alligators, though principally ureotelic, eliminate considerable amounts of ammonia also in their urine.

In higher animals, ammonia released by the deamination of amino acids is largely incorporated into glutamine as its amide group. Reaching the kidneys, glutamine is hydrolyzed by *glutaminase* to release the ammonia which is secreted in the urine.

Ureotelism

Excretion of urea as the principal nitrogenous endproduct, is known as ureotelism. Ureotelic animals include mammals, elasmobranchs, terrestrial and semi-aquatic amphibians like frogs and toads, and many aquatic

and semi-aquatic reptiles such as turtles, terrapins and alligators. These animals cannot eliminate ammonia sufficiently rapidly due to the limited availability of water, relatively smaller body surface area and poorer permeability of their integument. But they can still excrete enough water for eliminating urea which, being far less toxic than ammonia, can be excreted more slowly. So, these animals convert ammonia to urea in the liver. Mammals can concentrate urea considerably in the urine, thus losing far less water in its elimination. Elasmobranchs (e.g., sharks, saw fishes and sting rays) retain much urea in their body fluids (upto 300 mM litre⁻¹) by (i) minimizing the loss of urea through gill membranes and rectal glands, and (ii) actively reabsorbing urinary urea in renal tubules. This makes their body fluids hyperosmotic to the surrounding sea-water and prevents the osmotic loss of water through the body surface.

Some animals can temporarily switch over from ammonotelism to ureotelism when threatened with dehydration. Ammonotelic earthworm turns ureotelic in dry environment and during enhanced protein catabolism in starvation. African lung-fish *Protopterus*, normally ammonotelic, turns ureotelic and retains urea upto 1% of the body weight during aestivation in dry seasons. Ammonotelic *Xenopus* toads normally excrete only 20-30% of the urinary N as urea, but considerably increase the synthesis of urea and largely store it in the body when placed in saline water or brackish water. Such switch-overs minimize urinary loss of water in eliminating nitrogenous metabolites, and also elevate the plasma osmotic pressure to minimize water loss through the body surface. However, such fishes and amphibians cannot synthesize the required amount of urea from ammonia through the urea synthesis cycle alone; they synthesize some more urea through the catabolism of pyrimidines and

also by the cleavage of allantoic acid, a uric acid metabolite. On returning to normal conditions, these animals switch back to ammonotelism and eliminate the retained urea from the body.

Tadpoles switch over permanently from ammonotelism to ureotelism when they metamorphose into adult terrestrial toads or frogs.

Purinotelism and uricotelism

Purinotelism is the elimination of nitrogenous endproduct mainly as a purine like uric acid, guanine or xanthine. The term *uricotelism* is used if uric acid is the principal nitrogenous endproduct. Ammonia is converted to uric acid in the liver of terrestrial egg-laying and arid-zone animals to whom very limited amounts of water are available. Bats and birds have another handicap; they would overburden their flight if they carry as much water as is needed for ureotelism. Being far less toxic than ammonia and urea, uric acid may be allowed to reach higher concentrations than even urea in the body. Moreover, it is only sparingly soluble in water and its crystals need little water to be swept out as an insoluble white sludge in the urine. In the shelled eggs of reptiles, birds and insects, uric acid is retained as a solid non-toxic deposit, needing neither water nor a mechanism for its extrusion from the egg. Uricotelic animals include birds, most insects, land snails, land tortoises, lizards and some land crustaceans like crabs.

Ammonia from deamination of amino acids is incorporated as the amide group in glutamine. Transaminases transfer the amino groups of some amino acids to aspartate. Both the amide-N of glutamine and the amino-N of aspartate are used in synthesizing inosinic acid by the usual pathway for purine synthesis in the liver. Inosinic acid is then changed to uric acid by the usual hepatic pathway of purine catabolism. However, in

contrast to non-uricotelic animals, high protein intakes enhance the activities of glutamine synthase, glutamine PRPP amidotransferase and xanthine oxidase, and thereby increase the rates of glutamine synthesis, synthesis of inosinic acid and its catabolism to uric acid respectively. This may explain how the same pathways, existing in both uricotelic and non-uricotelic animals, may be utilized in the former in producing large amounts of uric acid from amino acid nitrogen.

Other purinotelic animals include arachnids such as spiders and scorpions which mainly eliminate guanine, and some uricotelic snails which excrete guanine and xanthine in addition to uric acid.

12.10 AMMONIA TOXICITY

Mammalian liver detoxicates most of the ammonia by changing it to urea. In conditions such as liver failure and inborn errors of urea synthesis, ammonia concentration rises in the blood (*hyperammonemia*) and in other tissues. This produces ammonia toxicity in many ways.

(a) Increased ammonia concentration manifold enhances the reductive amination of α -ketoglutarate to glutamate by glutamate dehydrogenase in the brain. This reduces the mitochondrial pool of α -ketoglutarate, consequently depressing the TCA cycle and cellular respiration of brain cells.

(b) Increased ammonia concentration also enhances the synthesis of glutamine from glutamate. It reduces the brain cell pool of glutamate, a neurotransmitter in the brain. It also decreases the availability of glutamate for the production of the inhibitory neurotransmitter γ -aminobutyrate.

(c) The rise in the brain glutamine level enhances the outflow of glutamine from brain cells. As glutamine is carried out by the same transporter which simultaneously carries tryptophan into the brain cells, the brain cell

pool of tryptophan is increased, leading to an abnormal increase in the synthesis of the neurotransmitter serotonin from tryptophan.

(d) NH_4^+ ions stimulate the secretion of glucagon which enhances renal gluconeogenesis from amino acids and consequently increases the ammonia load further.

Symptoms of ammonia toxicity include hyperammonemia, high urinary ammonia, and many neurological symptoms such as nausea, vomiting, dizziness, slurring of speech, tremor, ataxia, convulsions, blurring of vision, aversion to high-protein diet, lethargy, mental retardation and coma. It may end in death.

12.11 SYNTHESIS OF UREA

In ureotelic animals, ammonia released from amino acids is mostly detoxicated by conversion to urea.

Site of synthesis

Mann, Bolman and Magath showed that both blood and urinary levels of urea declined progressively and almost parallel to each other in hepatectomized dogs maintained by glucose infusion, but the blood amino acid level rose progressively. On the other hand, the blood urea rose progressively if a normal animal was nephrectomized; but the blood urea stopped rising further if the animal was now hepatectomized.

On incubation of different tissue slices with ammonium salts, bicarbonate, lactate (as a source of energy) and catalytic amounts of ornithine or arginine, only liver slices were found to produce significant amounts of urea.

Liver was found to contain the enzyme *arginase* which hydrolyzes arginine to urea and ornithine.

Such findings showed that urea is mostly synthesized in the liver. It has been shown subsequently that liver contains all the enzymes for synthesizing urea from ammonia and carbon dioxide. Kidneys can synthesize

arginine from ammonia, carbon dioxide and ornithine, but lack *arginase* for converting arginine to urea. Brain can synthesize urea from citrulline, but lacks the enzyme for forming citrulline from ornithine. Thus, neither the kidneys nor the brain can contribute significant amounts of urea to the body.

Reaction sequence

According to the *arginine-urea pathway* proposed by Krebs and Henseleit, liver condenses a molecule each of ammonia and carbon dioxide with ornithine to form citrulline, the latter is condensed with another molecule of ammonia to form arginine, and *arginase* finally hydrolyzes arginine to ornithine and urea (Fig. 12.11).

This original scheme has been extensively elucidated and modified by subsequent work (Fig. 12.12). Hepatic mitochondrial enzymes catalyze the initial steps of formation of citrulline from ammonia, carbon dioxide and ornithine; enzymes of hepatic cytosol catalyze the subsequent conversion of citrulline to arginine and of the latter to urea and ornithine.

1. Synthesis of carbamoyl phosphate:

In hepatic mitochondria, *carbamoyl phosphate synthase I* condenses ammonia, carbon dioxide and a phosphate group of ATP irreversibly into carbamoyl phosphate (Fig. 12.12). Two molecules of Mg^{2+} -ATP are hydrolyzed in this reaction to drive the endergonic synthesis of two bonds in carbamoyl phosphate, viz.,

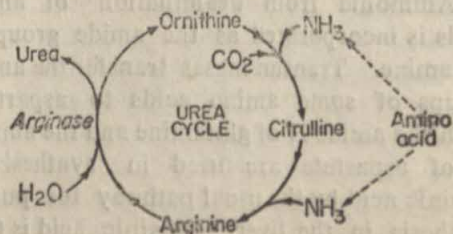


Fig. 12.11. Krebs and Henseleit's arginine-urea pathway or urea cycle.

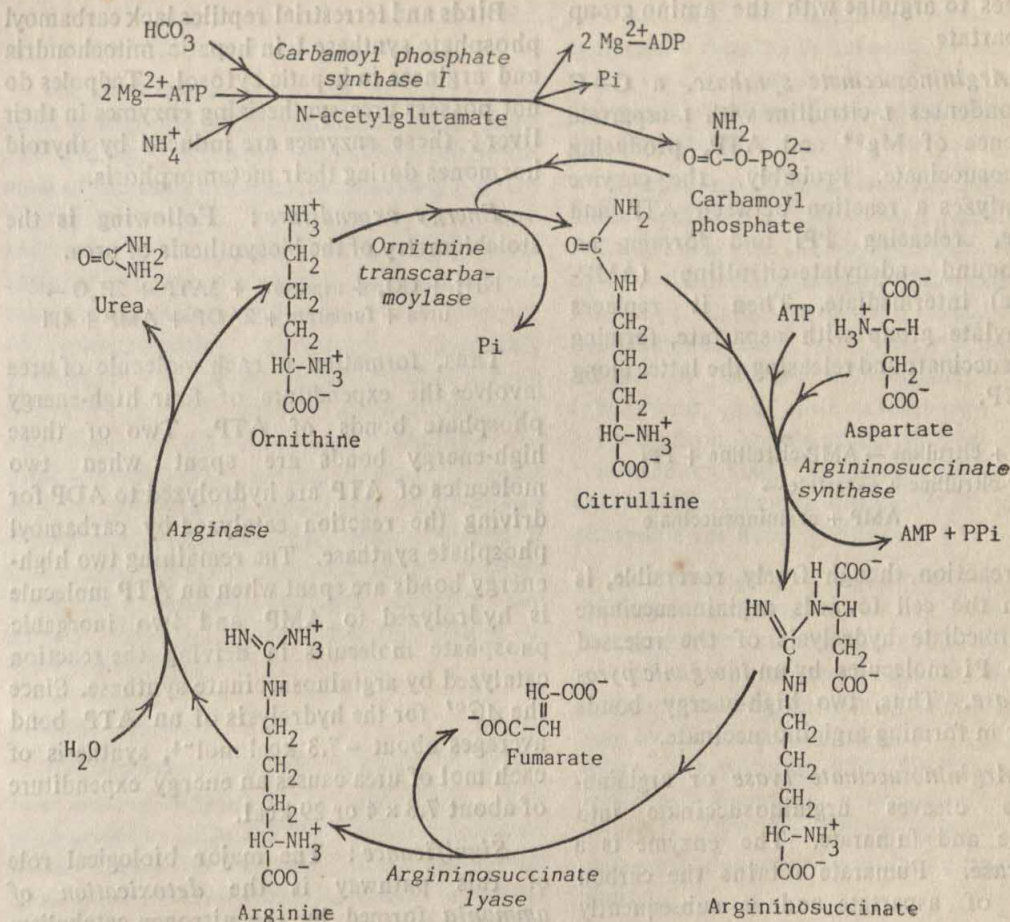


Fig. 12.12. Modern concept of the arginine-urea pathway.

an amide bond and a carboxylic-phosphoric anhydride bond. Carbamoyl phosphate synthase I is the *rate-limiting enzyme* of the urea cycle. In contrast to the cytoplasmic carbamoyl phosphate synthase II, this enzyme (i) occurs in the mitochondrial matrix, (ii) is closely associated there with ornithine transcarbamoylase, (iii) produces carbamoyl phosphate for utilization in urea formation and not for pyrimidine synthesis, (iv) has a low K_m for ammonia and utilizes free ammonia instead of glutamine in the reaction, and (v) requires N-acetylglutamate as the allosteric activator. N-acetylglutamate causes

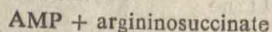
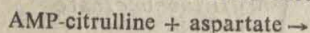
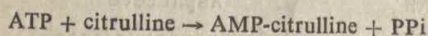
more than a fourfold increase in the activity of this enzyme by reducing its K_m for both the ATP molecules.

2. *Synthesis of citrulline*: The carbamoyl group is next transferred from carbamoyl phosphate to L-ornithine in hepatic mitochondria by *ornithine transcarbamoylase*. Ornithine is thereby changed to citrulline. The latter is then transferred to the cytosol across the inner membrane probably by a carrier of that membrane.

3. *Synthesis of arginine*: In the cytosol of liver cells, citrulline is further aminated in

two stages to arginine with the amino group from aspartate.

(a) *Argininosuccinate synthase*, a C—C ligase, condenses L-citrulline with L-aspartate in presence of Mg^{2+} and ATP, producing L-argininosuccinate. Probably, the enzyme first catalyzes a reaction between ATP and citrulline, releasing PPi and forming an enzyme-bound adenylate-citrulline (AMP-citrulline) intermediate. Then it replaces the adenylate group with aspartate, forming argininosuccinate and releasing the latter along with AMP.



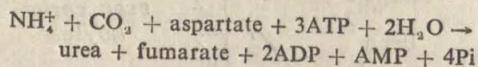
The reaction, though freely reversible, is driven in the cell towards argininosuccinate by the immediate hydrolysis of the released PPi into Pi molecules by an *inorganic pyrophosphatase*. Thus, two high-energy bonds are spent in forming argininosuccinate.

(b) *Argininosuccinate lyase* or argininosuccinase cleaves argininosuccinate into L-arginine and fumarate. The enzyme is a C—N lyase. Fumarate retains the carbon skeleton of aspartate and is subsequently changed through the TCA cycle to oxaloacetate; the latter is transaminated to aspartate to repeat its performance in the urea cycle. Thus, the nitrogen entering arginine from aspartate comes ultimately from other amino acids, particularly glutamate, through transamination.

4. *Conversion of arginine to urea*: In the hepatic cytosol of ureotelic animals, Mn^{2+} -dependent *arginase* hydrolyzes arginine to ornithine and urea. To repeat the cycle, ornithine is transported back into the mitochondrion probably by the same carrier of the inner membrane which carries citrulline out from the mitochondrial matrix to the cytosol.

Birds and terrestrial reptiles lack carbamoyl phosphate synthase I in hepatic mitochondria and arginase in hepatic cytosol. Tadpoles do not possess urea-synthesizing enzymes in their liver; these enzymes are induced by thyroid hormones during their metamorphosis.

Energy expenditure: Following is the stoichiometry of the biosynthesis of urea.



Thus, formation of each molecule of urea involves the expenditure of four high-energy phosphate bonds of ATP. Two of these high-energy bonds are spent when two molecules of ATP are hydrolyzed to ADP for driving the reaction catalyzed by carbamoyl phosphate synthase. The remaining two high-energy bonds are spent when an ATP molecule is hydrolyzed to AMP and two inorganic phosphate molecules in driving the reaction catalyzed by argininosuccinate synthase. Since the ΔG° for the hydrolysis of an ATP bond averages about $-7.3 \text{ kcal mol}^{-1}$, synthesis of each mol of urea causes an energy expenditure of about 7.3×4 or 29 kcal.

Significance: The major biological role of this pathway is the *detoxication of ammonia* formed by the nitrogen catabolism of amino acids. In an average normal man, about 12-14 g of urea-N is formed in 24 hours; this represents more than 80% of the total urinary nitrogen. A decline in urea synthesis due to any inborn error of urea cycle enzymes produces hyperammonemia and ammonia toxicity symptoms.

On a normal protein intake, urea is *mainly exogenous* in origin, being formed largely from dietary amino acids. On a protein-free diet with adequate calorie content in the form of carbohydrates and fats, urea is *endogenous* in origin, being formed from tissue proteins catabolized for synthesizing essential non-protein biomolecules such as heme, creatine and neurotransmitters. Endogenous urea

risers considerably in starvation because of the catabolism of tissue proteins for energy.

The urea cycle also serves for the *biosynthesis of arginine* from ornithine in both liver and kidney. Kidneys probably contribute most of the body arginine, because they possess all the urea cycle enzymes except arginase and can consequently run the cycle upto arginine but cannot cleave it to urea. Birds cannot synthesize arginine due to the absence of mitochondrial carbamoyl phosphate synthase I. In young mammals like human infants, the urea cycle has a limited capacity and fails to provide enough arginine for protein synthesis in growing tissues. Arginine is thus an essential amino acid for birds and young mammals. In the inborn errors of urea cycle enzymes, reduced tissue protein synthesis, negative nitrogen balance and growth failure result from the failure to synthesize arginine.

Regulation

Carbamoyl phosphate synthase I is the *rate-limiting enzyme* of the urea cycle. It is allosterically activated by N-acetylglutamate which lowers its K_m for ATP. According to the *Rubio-Britton-Grisolia model* for carbamoyl phosphate synthase I, the latter changes its conformation to an active form on binding with the first ATP molecule and further alters its conformation to a second active form on binding with N-acetylglutamate; this exposes specific SH groups and conceals others in the enzyme molecule and thereby increases the ATP-affinity of the enzyme; this promotes the transfer of a phosphate group from ATP to an enzyme-bound carbamate intermediate, changing the latter to carbamoyl phosphate. So, the liver content of N-acetylglutamate controls the rate of urea synthesis by allosterically regulating its rate-limiting enzyme. Arginine as well as food intake increases the synthesis and amount of N-acetylglutamate in the liver by activating N-acetylglutamate

synthase, and consequently enhances urea synthesis. Accumulation of carbamoyl phosphate, on the other hand, inhibits carbamoyl phosphate synthase I to reduce the rate of urea formation.

By utilizing free ammonia, carbamoyl phosphate synthase I lowers its concentration in mitochondria and thereby promotes the action of mitochondrial glutamate dehydrogenase in catalyzing further ammonia formation. The two enzymes thus act in a well-coordinated manner in hepatic mitochondria. On the other hand, the close association between carbamoyl phosphate synthase I and ornithine transcarbamoylase in hepatic mitochondria ensures the efficient utilization of carbamoyl phosphate for urea synthesis.

Increased aerobic metabolism through the TCA cycle promotes urea synthesis because the operation of the TCA cycle (i) lowers the mitochondrial concentration of α -ketoglutarate, thereby promoting ammonia formation by increased glutamate dehydrogenase action, (ii) provides ATP for the action of carbamoyl phosphate synthase and (iii) also furnishes ATP for allosterically activating glutamate dehydrogenase for ammonia formation.

Besides carbamoyl phosphate synthase I, ornithine transcarbamoylase and argininosuccinate synthase also undergo feed-back inhibition by their respective products, viz., citrulline and argininosuccinate. Thyroid hormones induce all three enzymes as well as arginase.

Inborn errors

These rare metabolic diseases result from the genetic deficiency of specific urea cycle enzymes. The substrate of the defective enzyme rises in the blood and urine; this causes a feed-back inhibition of the enzyme of the preceding step with accumulation of its substrate too. This is repeated stepwise and

ultimately inhibits carbamoyl phosphate synthase I, causing hyperammonemia and ammonia toxicity symptoms.

Hyperammonemia I: An inborn defect of carbamoyl phosphate synthase I produces hyperammonemia and symptoms of ammonia toxicity such as protein intolerance, ataxia and vomiting.

Ornithinemia (hyperammonemia II): This results from an X chromosome-linked deficiency of ornithine transcarbamoylase. Ammonia, ornithine and glutamine rise in blood, cerebrospinal fluid and urine. Ammonia toxicity symptoms appear.

Citrullinemia: Blood and urinary levels of citrulline rise due to either of two genetic defects of argininosuccinate synthase. In one case, caused probably by a mutation of its regulator gene, the enzyme has its normal K_m for citrulline, but is very poor in amount in the liver. In another case, probably due to a structural gene mutation affecting its catalytic site, the enzyme has an abnormally high K_m although its amount is normal in the liver. Hyperammonemia and mental retardation are seen in citrullinemia.

Argininosuccinic aciduria: This fatal disease is characterized by high argininosuccinate levels in blood, urine and cerebrospinal fluid, hyperammonemia, mental retardation and friable tufted hair. It results from a genetic deficiency of argininosuccinate lyase.

Argininemia: Arginase is deficient in liver and erythrocytes. Arginine level rises in blood, CSF and brain. Hyperammonemia is common. Urinary levels of lysine, cystine, ornithine and arginine are elevated.

12.12 GLUTAMINE AND TRANSAMIDATION

Some of the ammonia, freed from amino acids, is rapidly converted to glutamine. This is another process of ammonia detoxication in many tissues. It allows ammonia to be transported as non-toxic glutamine to the liver and kidneys from peripheral tissues for further metabolism.

Synthesis of glutamine:

Glutamine is synthesized by *glutamine synthase*, a mitochondrial C-N ligase of particularly renal cells. The enzyme first phosphorylates the γ COOH of L-glutamate with the help of ATP to produce an enzyme-bound γ -glutamyl phosphate intermediate (Fig. 12.13). The phosphate of this γ carboxylic phosphoric anhydride is then replaced by free ammonia, producing and releasing glutamine.

Glutamine synthase (GS) is largely regulated by reversible adenylation and deadenylation. In *E. coli*, inactive GS_b is changed to active GS_a by an Mn^{2+} -dependent *deadenylase* which removes 12 adenylate groups from GS_b by phosphorolysis (Fig. 12.14); GS_a is adenylated to inactive GS_b by an Mg^{2+} -dependent *GS_a adenyltransferase* and 12 ATP

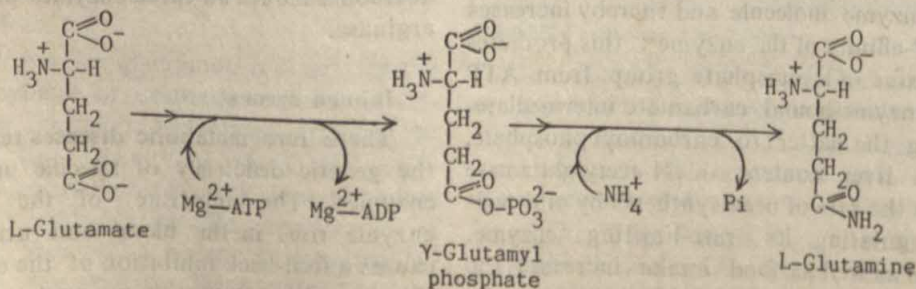


Fig. 12.13. Synthesis of glutamine.

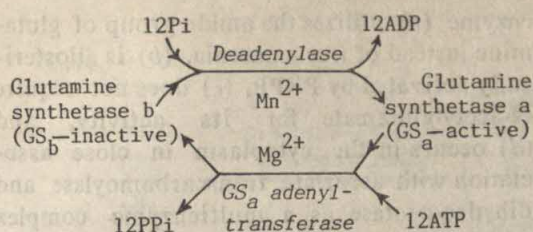


Fig. 12.14. Regulation of glutamine synthase in *E. coli*.

molecules (*multisite adenylation*). Glutamine inhibits the deadenylyase and activates the adenylyltransferase to reduce further glutamine synthesis. In *E. coli*, the same enzyme exerts both deadenylyase and adenylyltransferase actions, depending on its binding with respectively the uridylylated (PD) and deuridylylated (PA) forms of a regulatory protein. Glutamine synthesis is also reduced by the feed-back inhibition of glutamine synthase by carbamoyl

phosphate, glucosamine 6-phosphate, AMP and CTP, all formed with the help of glutamine.

Transamidation:

Glutamine donates its amide-N to many substrates under the action of specific *amidotransferases*.

(i) *L-Glutamine D-fructose 6-phosphate amidotransferase* transfers the γ -amide group of glutamine to the C^2 of fructose 6-phosphate, changing the latter to glucosamine 6-phosphate (page 351).

(ii) *Glutamine PRPP amidotransferase* catalyzes the very first and rate-limiting step of purine synthesis. It transfers the amide group of glutamine to 5-phosphoribosyl-1-pyrophosphate (PRPP), changing the latter to 5-phosphoribosyl-1-amine (Fig. 12.15).

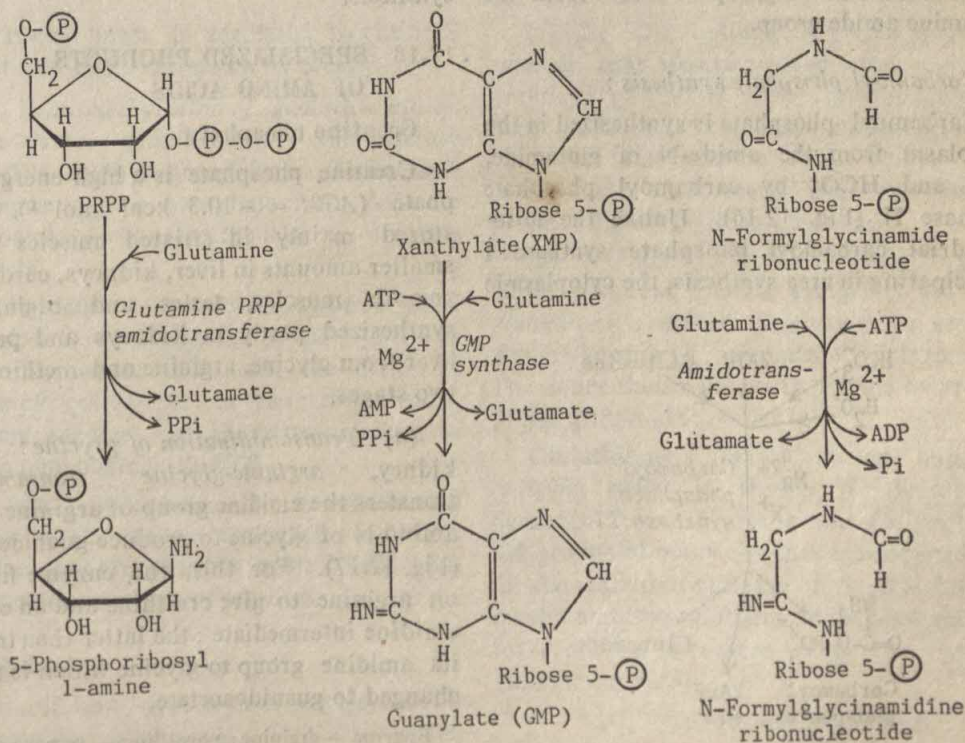
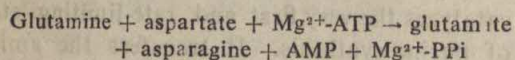


Fig. 12.15. Some transamidation reactions.

(iii) N-Formylglycinamide ribonucleotide, an intermediate in purine synthesis, undergoes transamidation with the glutamine amide group to form N-formylglycinamide ribonucleotide.

(iv) *GMP synthase* transfers the amide group of glutamine to the C² of xanthylate (XMP), changing it to guanylate (GMP).

(v) *Asparagine synthase* transfers the amide group of glutamine to the β -COOH group of aspartate, using ATP as a co-substrate. Aspartate is thereby changed to asparagine while ATP is cleaved to AMP and PPi.



Reactions (ii) and (iii) incorporate the amide-N of glutamine at respectively N⁹ and N³ of the purine ring while reaction (iv) gives rise to the C²-amino group of GMP from the glutamine amide group.

Carbamoyl phosphate synthesis :

Carbamoyl phosphate is synthesized in the cytoplasm from the amide-N of glutamine, ATP and HCO₃⁻ by carbamoyl phosphate synthase II (Fig. 12.16). Unlike the mitochondrial carbamoyl phosphate synthase I participating in urea synthesis, the cytoplasmic

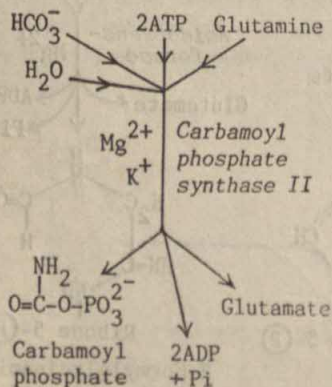


Fig. 12.16. Synthesis of carbamoyl phosphate from glutamine.

enzyme (a) utilizes the amide group of glutamine instead of free ammonia, (b) is allosterically activated by PRPP, (c) does not require N-acetylglutamate for its activity, and (d) occurs in the cytoplasm in close association with aspartate transcarbamoylase and dihydro-orotase as a multienzyme complex which catalyzes the first three steps of pyrimidine synthesis. Through the carbamoyl group of carbamoyl phosphate, the amide-N of glutamine goes to form the N³ of the pyrimidine ring.

Hydrolysis :

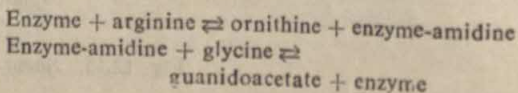
Glutamine is hydrolyzed in renal and hepatic mitochondria into glutamate and ammonia by different glutaminase isoenzymes (page 363). This ammonia is then either excreted into renal tubules for buffering urinary acids or used in the liver for urea synthesis.

12.13 SPECIALIZED PRODUCTS OF AMINO ACIDS

Creatine phosphate

Creatine phosphate is a high-energy phosphate ($\Delta G^{\circ'} = -10.3 \text{ kcal mol}^{-1}$). It is stored mainly in striated muscles and in smaller amounts in liver, kidneys, cardiac and smooth muscles, testes and brain. It is synthesized partly in kidneys and partly in liver from glycine, arginine and methionine in two stages.

(a) *Transamidation of glycine* : In the kidney, *arginine-glycine transaminidase* transfers the amidine group of arginine to the amino-N of glycine to produce guanidoacetate (Fig. 12.17). For this, the enzyme first acts on arginine to give ornithine and an enzyme-amidine intermediate ; the latter then transfers its amidine group to glycine which is thereby changed to guanidoacetate.



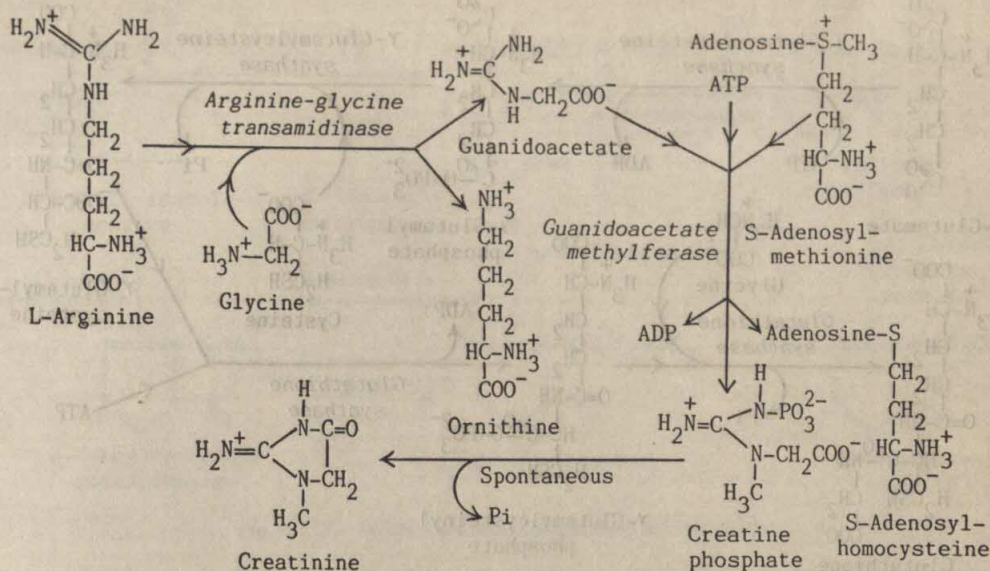


Fig. 12.17. Synthesis and catabolism of creatine phosphate.

The transaminidase is regulated by the end-product feed-back repression by creatine.

(b) *Transmethylation of guanidoacetate*: In the liver, *guanidoacetate methyltransferase* transfers a methyl group from S-adenosyl-methionine and a phosphate from ATP to guanidoacetate, changing the latter to creatine phosphate.

During muscle contraction, creatine phosphate donates its high-energy phosphate to ADP to regenerate ATP under the action of *creatine phosphokinase*. In the resting muscle, the same enzyme phosphorylates creatine to creatine phosphate with ATP.

Creatine phosphate is spontaneously changed in the muscle to Pi and creatinine. The latter is excreted in the urine along with much smaller amounts of creatine.

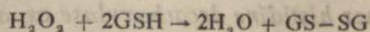
Glutathione

Glutathione (γ -glutamylcysteinylglycine) is a tripeptide synthesized from glutamate, cysteine and glycine in the cytosol without any participation of RNA.

(a) In the cytosol, γ -glutamylcysteine synthase first phosphorylates the γ -COOH group of glutamate with a phosphate group from ATP to form an enzyme-glutamyl phosphate intermediate (Fig. 12.18). The intermediate then reacts with cysteine to produce Pi and γ -glutamylcysteine.

(b) γ -Glutamylcysteine is phosphorylated at its cysteinyl COOH group by ATP and *glutathione synthase*, producing an enzyme-glutamylcysteinyl phosphate intermediate. The latter then reacts with glycine to produce Pi and glutathione.

Glutathione (GSH) is an important *reducing agent* in tissues. It donates the hydrogen from its SH group for protecting SH groups of many enzymes against oxidation, for the reductive cleavage of S—S linkages in insulin and thyroglobulin molecules and also for reducing peroxides with the help of *glutathione peroxidase*.



On donating hydrogen, two glutathione molecules get linked by an S—S linkage to give

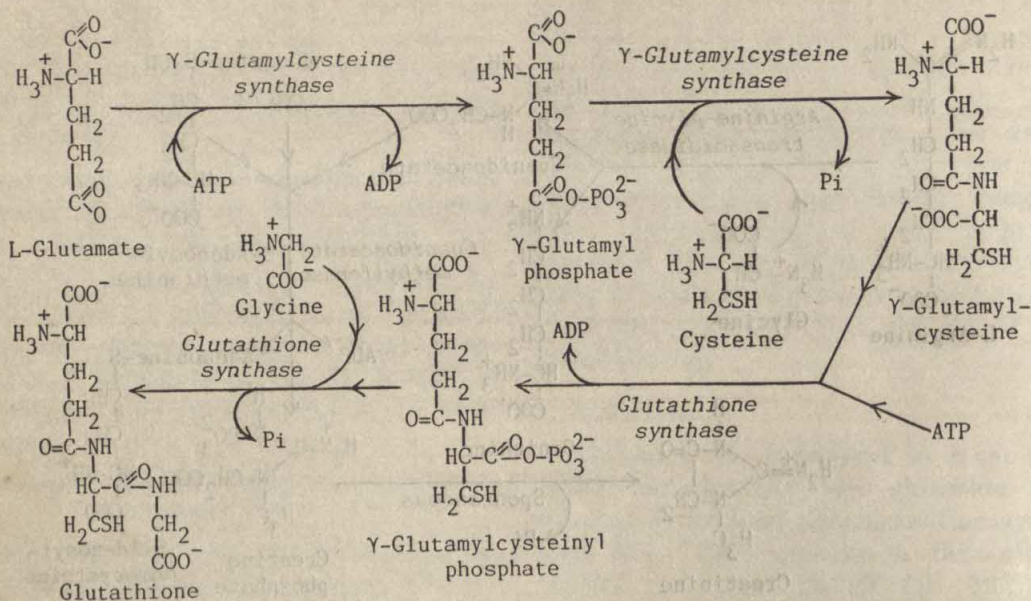
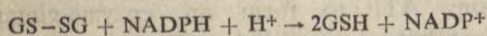


Fig. 12.18. Synthesis of glutathione.

oxidized glutathione (GS—SG). *Glutathione reductase* reduces the latter with the help of NADPH.



Glutathione is a cofactor for *maleylacetoacetate isomerase* which catalyzes the *cis-trans* isomerization of maleylacetoacetate to fumarylacetoacetate during tyrosine catabolism. It is also a cofactor for *prostaglandin endoperoxide isomerases* which isomerize PGH_2 to PGE_2 or PGD_2 .

Glutathione donates its cysteine moiety for conjugation with halogenated aromatic hydrocarbons during their detoxication to *mercapturic acids* (page 204).

The cysteine residue of glutathione is also used in the synthesis of the hair pigment *pheomelanin*.

Histamine

Either *histidine decarboxylase* or *aromatic L-amino acid decarboxylase* decarboxylates histidine to histamine in many mammalian

cells, particularly mast cells, gastric mucosa cells and histaminergic neurons of the central nervous system. These enzymes are C—C lyases, cleaving the bond between the carboxyl-C and the α -C of histidine (Fig. 12.19).

Histamine acts as a *neurotransmitter* in the central nervous system, particularly in the hypothalamus. It acts as an *anaphylactic and inflammatory agent*, on being released from mast cells in response to antigens (see page 238); on binding with H_1 receptors on blood vessels and smooth muscles, it causes vasodilatation, increased vascular permeability and spasms of smooth muscles—these anaphylactic reactions are minimized by pharmacological agents such as promethazine and mepyramine blocking H_1 receptors. Histamine also *stimulates gastric acid secretion* by binding with H_2 receptors on the gastric mucosa. It mediates in the effects of both gastrin and vagal impulses on gastric secretion. Blockers of H_2 receptors such as cimetidine are effective in reducing gastric acidity in peptic ulcer patients.

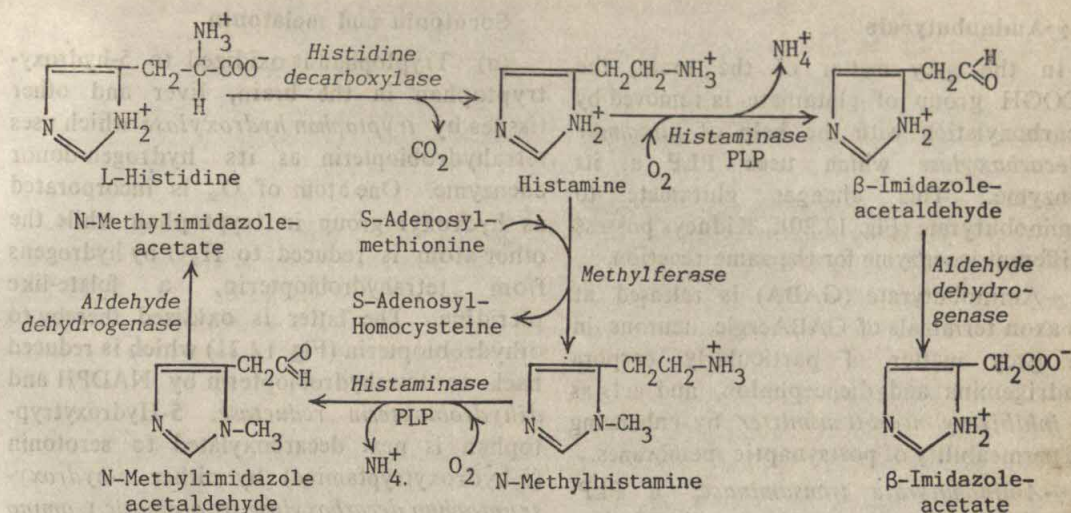


Fig. 12.19. Formation and catabolism of histamine.

Histamine is oxidatively deaminated to β -imidazole-acetaldehyde in kidneys by *histaminase*, using pyridoxal phosphate (PLP) as the coenzyme (Fig. 12.19). The product is further oxidized by *aldehyde dehydrogenase* to imidazoleacetate which is excreted in the urine after conjugation with ribose. Alter-

natively, histamine is N-methylated in the liver with the help of a *methyltransferase* and S-adenosylmethionine to form N-methylhistamine; the latter is then changed to methylimidazoleacetate by histaminase and aldehyde dehydrogenase in the same way as histamine itself. The product is excreted.

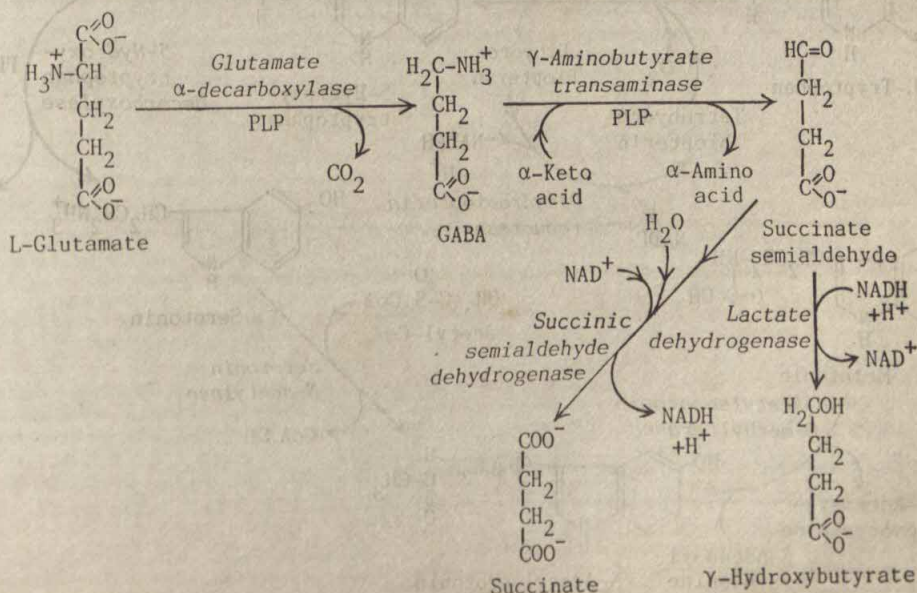


Fig. 12.20. Formation and catabolism of GABA.

γ -Aminobutyrate

In the grey matter of the brain, the α -COOH group of glutamate is removed by decarboxylation with the help of *glutamate α -decarboxylase* which uses PLP as its coenzyme. This changes glutamate to γ -aminobutyrate (Fig. 12.20). Kidneys possess a different isoenzyme for the same reaction.

γ -Aminobutyrate (GABA) is released at the axon terminals of GABAergic neurons in the grey matter of particularly corpora quadrigemina and diencephalon, and acts as an *inhibitory neurotransmitter* by enhancing K^+ permeability of postsynaptic membranes.

γ -Aminobutyrate transaminase, a PLP-dependent enzyme, transaminates GABA into inactive succinate semialdehyde. The latter is either oxidized to succinate by NAD^+ and *succinic semialdehyde dehydrogenase* or reduced to γ -hydroxybutyrate by NADH and *lactate dehydrogenase* (Fig. 12.20).

Serotonin and melatonin

(a) Tryptophan is oxidized to 5-hydroxytryptophan in the brain, liver and other tissues by *tryptophan hydroxylase* which uses tetrahydrobiopterin as its hydrogen-donor coenzyme. One atom of O_2 is incorporated as hydroxyl group in tryptophan while the other atom is reduced to H_2O by hydrogens from tetrahydrobiopterin, a folate-like pteridine. The latter is oxidized thereby to dihydrobiopterin (Fig. 12.21) which is reduced back to tetrahydrobiopterin by NADPH and *dihydrobiopterin reductase*. 5-Hydroxytryptophan is next decarboxylated to serotonin (5-hydroxytryptamine) by either *5-hydroxytryptophan decarboxylase* or *aromatic L-amino acid decarboxylase*, both PLP-dependent enzymes.

Serotonin is synthesized in the brain, liver, kidneys and gastrointestinal tract. It is collected, stored and released from blood

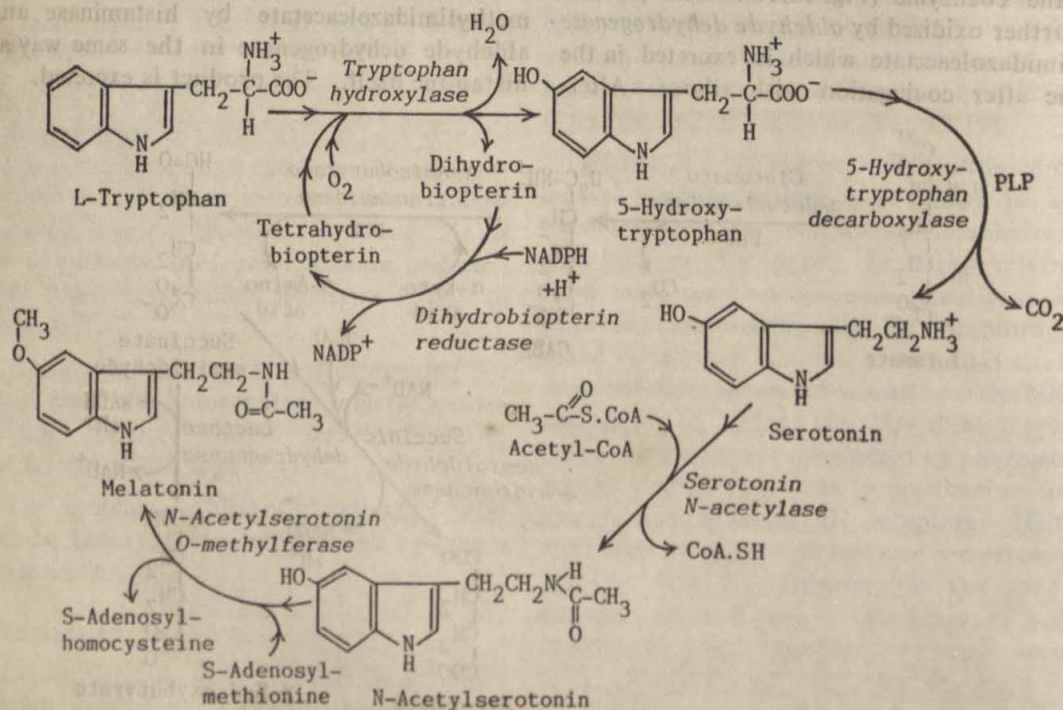


Fig. 12.21. Synthesis of serotonin and melatonin.

platelets. It is an important *neurotransmitter* released at the axon terminals of serotonergic neurons of hypothalamus and other parts of the brain. Serotonin is a *vasoactive amine* and may cause vasoconstriction; it also causes contraction of smooth muscles, stimulation of respiratory and circulatory chemoreceptors and pain nerve endings, tachycardia, rise of BP and hyperpnea. It is oxidatively deaminated by *monoamine oxidase* and then oxidized by *aldehyde dehydrogenase* to 5-hydroxyindoleacetate which is excreted in the urine after detoxication in the liver by conjugation.

Decreased serotonin synthesis produces neurological symptoms in an inborn disorder called *Hartnup disease*. In this disease, the membrane transporter for large neutral amino acids is genetically defective in intestinal mucosa, renal tubules and probably the brain. This reduces their renal reabsorption and causes high urinary elimination of tryptophan, other neutral amino acids and abnormal tryptophan metabolites like indoleacetate, poor circulating levels of tryptophan and other amino acids, impaired synthesis of serotonin and nicotinamide (both tryptophan-products), intermittent ataxia, mental retardation and pellagra-like symptoms such as cutaneous hypersensitivity to sunlight.

(b) In the pineal gland, serotonin is N-acetylated to N-acetylserotonin by acetyl-CoA and *serotonin N-acetylase*. The 5-hydroxyl group of N-acetylserotonin is then methylated by *acetylserotonin O-methyltransferase*, using the labile methyl group of S-adenosylmethionine (Fig. 12.21). This produces melatonin. Serotonin N-acetylase is the *rate-limiting enzyme* in this process. Both synthesis and secretion of melatonin by the pineal gland are regulated by light. Melatonin participates in the production of *diurnal biological rhythms*. It may also mediate in the effect of light on seasonal reproductive cycles.

Catecholamines

Catecholamines include *adrenaline*, *noradrenaline* and *dopamine*. All three are synthesized from tyrosine and act as *neurotransmitters*—adrenaline and noradrenaline are released at axon terminals of adrenergic sympathetic fibres while dopamine is released at dopaminergic nerve terminals in particularly hypothalamus and diencephalon. Besides, adrenaline and noradrenaline are *adrenal medullary hormones*; dopamine is their precursor.

(a) Tyrosine is hydroxylated to 3,4-dihydroxyphenylalanine (DOPA) in adrenal chromaffin cells as well as adrenergic and dopaminergic neurons by *tyrosine hydroxylase*, using tetrahydrobiopterin as the hydrogen-donor coenzyme. By donating hydrogens to one atom of O_2 molecule to produce H_2O in this reaction, the coenzyme gets oxidized to dihydrobiopterin which is immediately reduced to tetrahydrobiopterin by NADPH and dihydrobiopterin reductase. Tyrosine hydroxylase is the *rate-limiting enzyme* for catecholamine synthesis (Fig. 12.22).

(b) Dopa is decarboxylated to dopamine by PLP-dependent *dopa decarboxylase* or *aromatic L-amino acid decarboxylase*.

(c) In adrenergic neurons and adrenal chromaffin cells, dopamine is hydroxylated to noradrenaline by *dopamine β -hydroxylase*, a copper-enzyme requiring L-ascorbate. The Cu^+ ion of the enzyme is oxidized to Cu^{2+} during catalysis and must be reduced back to Cu^+ by L-ascorbate.

(d) *Phenylethanolamine N-methyltransferase* uses the labile methyl group of S-adenosylmethionine to N-methylate noradrenaline to adrenaline. This enzyme undergoes feed-back inhibition by adrenaline.

Catecholamines are speedily inactivated by methylation at the ortho-hydroxyl group by *catecholamine O-methyltransferase* and S-adenosylmethionine, either followed or preceded

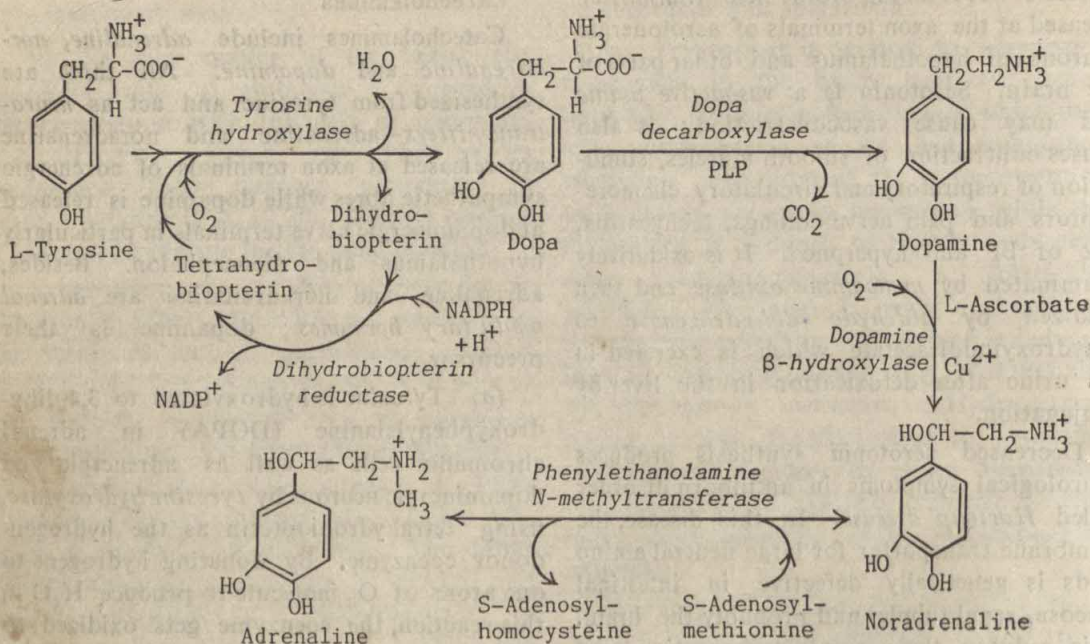


Fig. 12.22. Synthesis of catecholamines.

by oxidative deamination catalyzed by *monoamine oxidase* and *aldehyde dehydrogenase*. Endproducts such as vanilmandelic acid are excreted in the urine in conjugated and unconjugated forms.

Melanins

These are protein-bound polymeric pigments present in skin melanocytes, hair, iris, choroid, retinal pigmented cells and substantia nigra. Varying proportions of black *eumelanins* and red or brown *pheomelanins* occur in melanin polymers. They are synthesized in membrane-bound vesicles (melanosomes) in melanocytes.

(a) A copper-enzyme *tyrosinase* of melanocytes oxidizes tyrosine to dopa which is further oxidized to dopaquinone (Fig. 12.23). Tyrosinase is the *rate-limiting* enzyme of melanin biosynthesis. It is not known how far tyrosinase or any other enzyme is directly involved in the following reactions.

(b) Dopaquinone is cyclized to leucodopachrome which is then oxidized to red-colored dopachrome (hallachrome). The latter is decarboxylated to 5,6-dihydroxyindole which may be oxidized further to indole 5-6-quinone. These intermediates polymerize in presence of sunlight to form eumelanin heteropolymers.

(c) Dopaquinone may alternatively condense with the cysteinyl group from glutathione to produce 5-S-cysteinyl-dopa. The latter may ultimately give rise to benzothiazine intermediates which join to form pheomelanin heteropolymers.

In the substantia nigra, melanin is synthesized from dopamine instead of dopa or dopaquinone, by a process independent of tyrosinase.

Albinism :

Inherited deficiencies of melanin cause different forms of albinism characterized by melanomas and the lack of pigmentation of

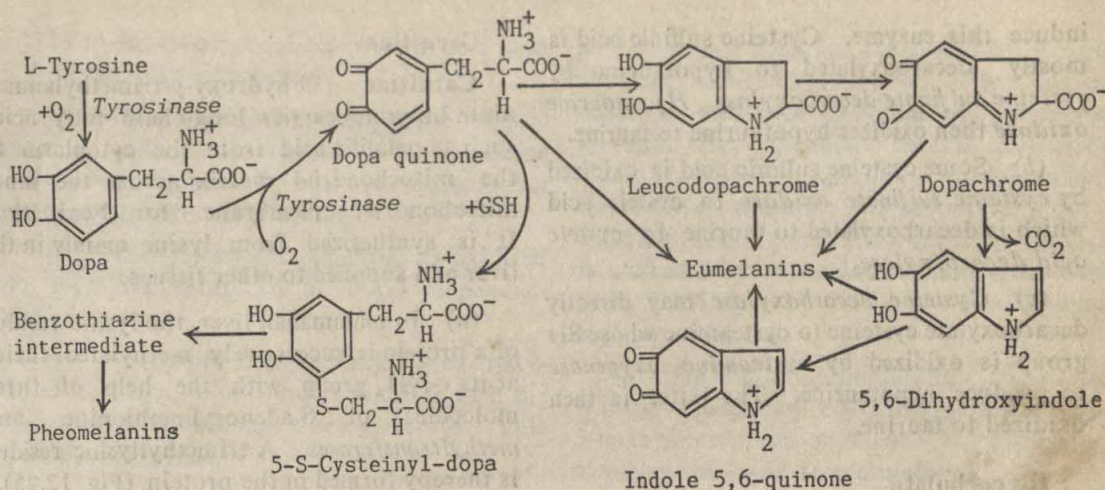


Fig. 12.23. Synthesis of melanins.

skin, hair and/or eyes. *Oculocutaneous albinism* occurs mostly as an autosomal recessive trait. Both skin and eyes lack pigmentation. In many cases, melanin is totally absent due to an inherited defect of tyrosinase. But in some cases, the defect lies somewhere else in melanin synthesis and some light pigmentation is visible. *Ocular albinism* affects retinal pigmentation. It may be inherited as either an X-linked or an autosomal recessive trait, and results from unknown metabolic defects.

Taurine

It is an oxidative metabolite of cysteine. Taurine is mainly conjugated with cholesteryl-CoA in the liver to form *taurocholate*, an important bile acid. Taurine probably functions also as a *neurotransmitter* in the retina and the central nervous system. Three alternative routes of taurine synthesis are given below in a descending order of their quantitative importance.

(a) *Cysteine oxidase* oxidizes the SH group of cysteine, changing it to cysteine sulfinic acid (Fig. 12.24); cysteine seems to,

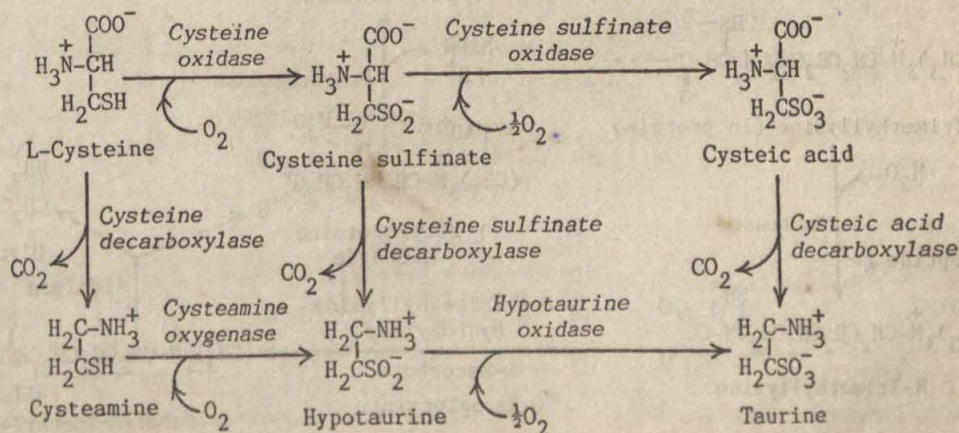


Fig. 12.24. Synthesis of taurine.

induce this enzyme. Cysteine sulfinic acid is mostly decarboxylated to hypotaurine by *cysteine sulfinic acid decarboxylase*. Hypotaurine oxidase then oxidizes hypotaurine to taurine.

(b) Some cysteine sulfinic acid is oxidized by *cysteine sulfinic acid oxidase* to cysteic acid which is decarboxylated to taurine by *cysteic acid decarboxylase*.

(c) *Cysteine decarboxylase* may directly decarboxylate cysteine to cysteamine whose SH group is oxidized by *cysteamine oxygenase* to produce hypotaurine. The latter is then oxidized to taurine.

Glycocholate

Similar to the synthesis of taurocholate from taurine, the bile acid glycocholate is synthesized by the conjugation of glycine with cheryl-CoA.

Carnitine

Carnitine (β -hydroxy- γ -trimethylammonium butyrate) carries long-chain fatty acids such as palmitic acid from the cytoplasm to the mitochondrial matrix across the inner mitochondrial membrane for β -oxidation. It is synthesized from lysine mainly in the liver and supplied to other tissues.

(a) In mammalian liver, the lysine residue of a protein is successively methylated thrice at its ϵ -NH₂ group with the help of three molecules of S-adenosylmethionine and *methyltransferases*. A trimethyllysine residue is thereby formed in the protein (Fig. 12.25).

(b) The ϵ -N-trimethyllysine, released by proteolysis of the protein, undergoes hydroxylation at its β -carbon to form ϵ -N-trimethyl- β -hydroxylysine with the help of ϵ -N-trimethyl-

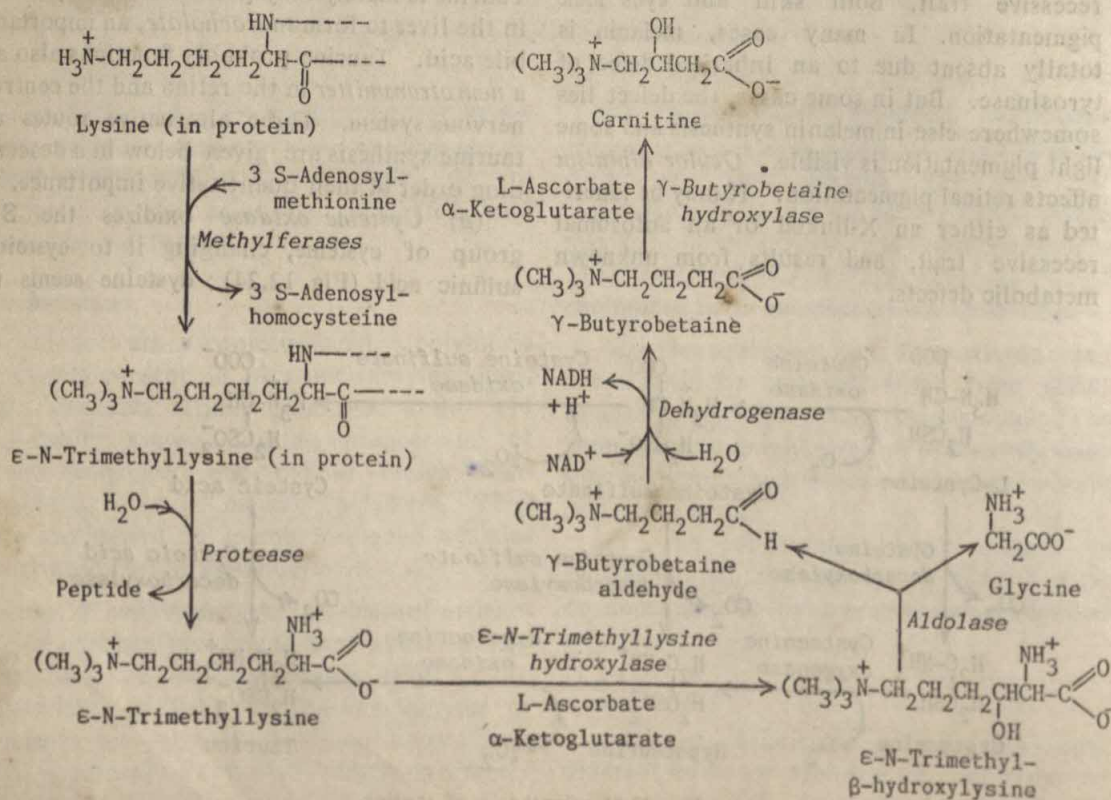


Fig. 12.25. Synthesis of carnitine.

lysine hydroxylase requiring L-ascorbate and α -ketoglutarate as cofactors.

(c) ϵ -N-trimethyl- β -hydroxylysine undergoes an aldol cleavage to γ -butyrobetaine aldehyde and glycine by an *aldolase* (probably *serine transhydroxymethylase*).

(d) γ -Butyrobetaine aldehyde is oxidized to γ -butyrobetaine by an NAD^+ -dependent *dehydrogenase*.

(e) γ -Butyrobetaine hydroxylase hydroxylates the β -carbon of γ -butyrobetaine with the help of L-ascorbate and α -ketoglutarate. This produces carnitine.

Polyamines

The polyamines such as putrescine, spermine and spermidine are synthesized from ornithine and S-adenosylmethionine.

(a) Ornithine and S-adenosylmethionine are decarboxylated by *ornithine decarboxylase* and *S-adenosylmethionine decarboxylase* respectively. The respective products are putrescine and S-adenosyl-3-methylmercaptopyrrolamine (Fig. 12.26). Ornithine decarboxylase is the *rate-limiting enzyme* for

polyamine synthesis and is induced by growth hormone and testosterone. The enzyme undergoes feed-back inhibition by putrescine, spermine and spermidine because these substances induce an inhibitory protein which inhibits the enzyme. S-Adenosylmethionine decarboxylase, a pyruvate-containing enzyme, is activated by putrescine and undergoes feed-back inhibition by S-adenosyl-3-methylmercaptopyrrolamine.

(b) *Putrescine propylamine transferase* transfers the aminopropyl group of S-adenosyl-3-methylmercaptopyrrolamine to putrescine, changing the latter to spermidine.

(c) *Spermidine propylamine transferase* transfers the aminopropyl group from a second S-adenosyl-3-methylmercaptopyrrolamine molecule to spermidine, changing the latter to spermine.

Polyamines act as growth factors in stimulating cell proliferation during growth and regeneration of tissues. They remain associated with nucleic acids, particularly RNA, and may stimulate their synthesis. They also play some roles in body immunity.

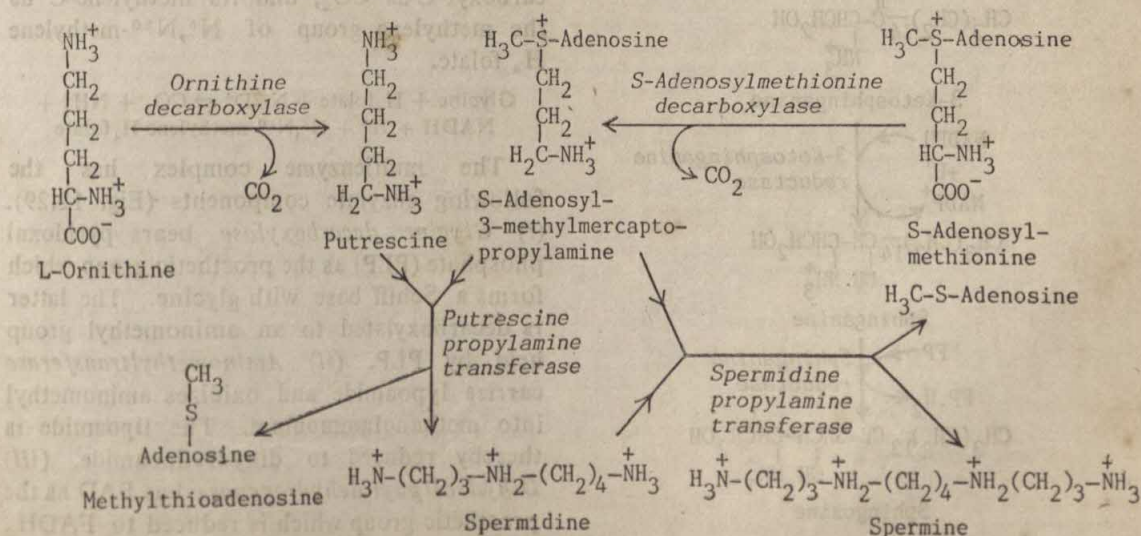


Fig. 12.26. Synthesis of polyamines.

Sphingosine

This essential constituent of sphingolipids is synthesized from serine and palmitic acid (Fig. 12.27). In the endoplasmic reticulum of brain and liver, serine first forms a Schiff base with the PLP prosthetic group of *3-ketosphinganine synthase*. Serine then loses CO_2 and condenses with palmitoyl group from palmitoyl-CoA to form *3-ketosphinganine*. The latter is reduced by NADPH-dependent *3-ketosphinganine reductase* into sphinganine which is oxidized to sphingosine by *sphinganine reductase*, a flavoprotein.

Hippuric acid

This is produced by the conjugation of benzoic acid with glycine in the liver. Benzoic

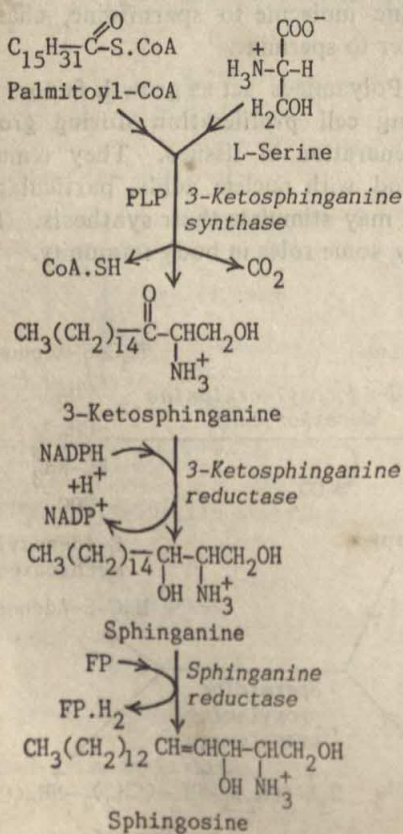


Fig. 12.27. Synthesis of sphingosine.

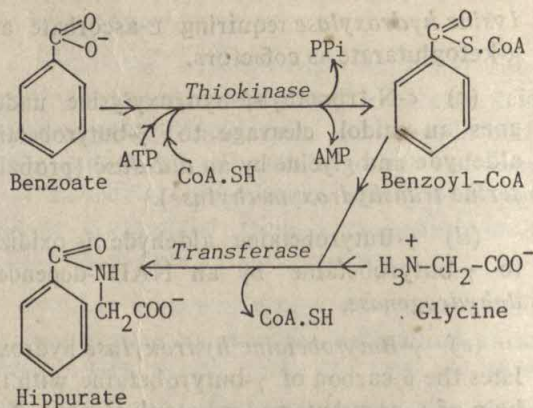


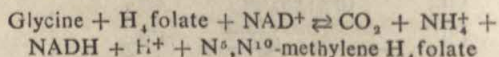
Fig. 12.28. Synthesis of hippurate.

acid is thioesterified to benzoyl-CoA by a *thiokinase*, utilizing ATP and coenzyme A (Fig. 12.28). The benzoyl group is then transferred to glycine to produce hippurate which is excreted in the urine.

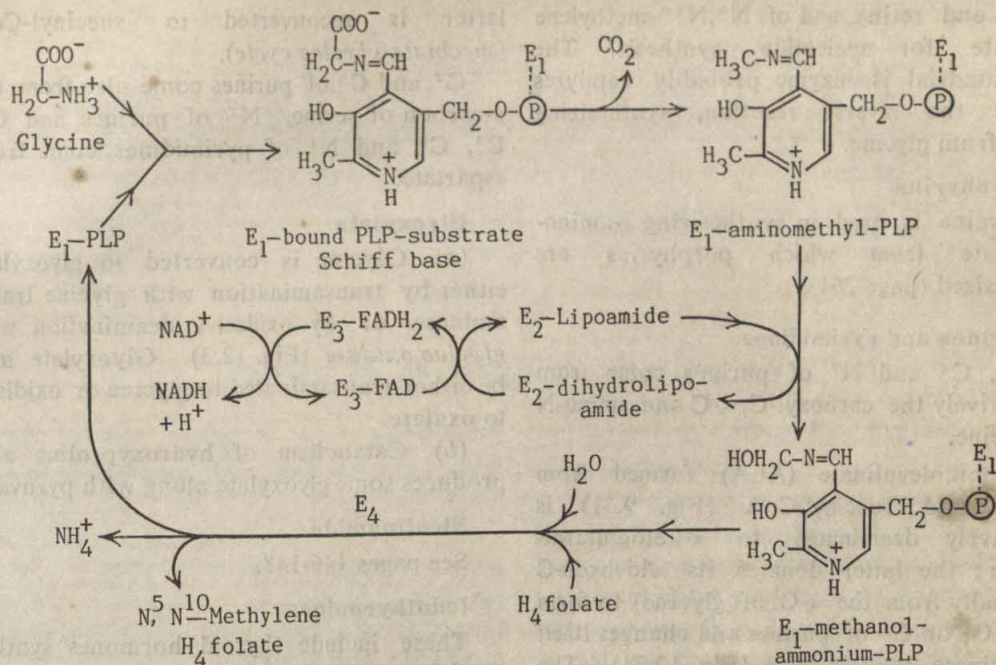
One-carbon compounds

Glycine and serine are important sources of C_1 compounds in the tissues.

(a) *Glycine cleavage system*: This mitochondrial multienzyme system cleaves glycine to release its amino group as ammonia, its carboxyl-C as CO_2 , and its methylene-C as the methylene group of $\text{N}^5, \text{N}^{10}$ -methylene H_4 folate.



The multienzyme complex has the following enzymic components (Fig. 12.29). (i) *Glycine decarboxylase* bears pyridoxal phosphate (PLP) as the prosthetic group which forms a Schiff base with glycine. The latter is decarboxylated to an aminomethyl group held by PLP. (ii) *Aminomethyltransferase* carries lipoamide and oxidizes aminomethyl into methanolammonium. The lipoamide is thereby reduced to dihydrolipoamide. (iii) *Dihydrolipoyl dehydrogenase* has FAD as the prosthetic group which is reduced to FADH_2 as the enzyme reoxidizes dihydrolipoamide



E_1 : Glycine decarboxylase. E_2 : Aminomethyl transferase.
 E_3 : Dihydrolipoyl dehydrogenase.
 E_4 : N^5, N^{10} -Methylene H_4 folate synthesizing protein.

Fig. 12.29. Mode of action of glycine cleavage enzyme

to lipoamide. $FADH_2$ is in turn reoxidized to FAD by NAD^+ . (iv) From the PLP-bound methanolammonium group, NH_4^+ is released and the carbon of the group is transferred to H_4 folate to form N^5, N^{10} -methylene H_4 folate with the help of an N^5, N^{10} -methylene H_4 folate synthesizing protein.

The enzyme system also acts as *glycine synthase* to form glycine by the reverse reaction from CO_2 , NH_4^+ and N^5, N^{10} -methylene H_4 folate.

(b) *Serine transhydroxymethylase*: In the cytosol, this PLP-containing enzyme catalyzes the aldol cleavage of serine into glycine, releasing the β -C of serine as the methylene group of N^5, N^{10} -methylene H_4 folate (Fig. 12.30). This is a major source of glycine as a neurotransmitter in the central nervous

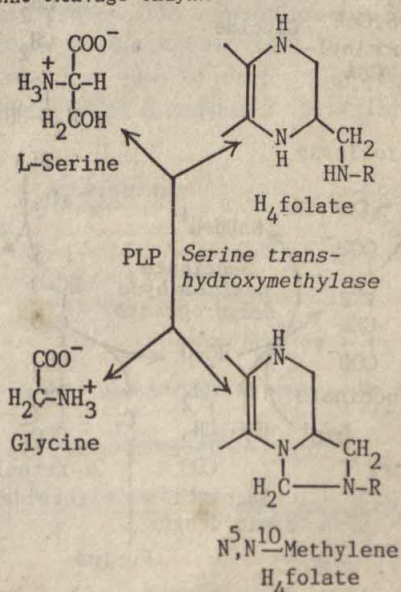


Fig. 12.30. Action of serine transhydroxymethylase.

system and retina, and of N^5, N^{10} -methylene H_4 folate for nucleotide synthesis. The mitochondrial isoenzyme probably catalyzes mainly the reverse reaction, synthesizing serine from glycine.

Porphyrins

Glycine is used in synthesizing δ -amino-levulinate from which porphyrins are synthesized (page 261).

Purines and pyrimidines

C^4 , C^5 and N^7 of purines come from respectively the carboxyl-C, α -C and amino-N of glycine.

δ -Aminolevulinate (ALA) formed from glycine and succinyl-CoA (Fig. 9.31) is oxidatively deaminated to α -ketoglutaraldehyde; the latter donates its aldehyde-C (originally from the α -C of glycine) to form either C^2 or C^8 of purines and changes itself to succinate semialdehyde (Fig. 12.31). The

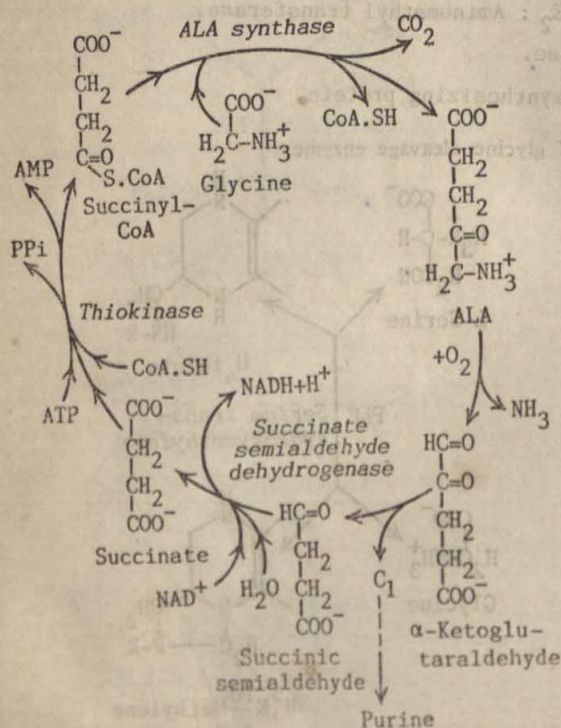


Fig. 12.31. Succinate-glycine cycle.

latter is reconverted to succinyl-CoA (succinate-glycine cycle).

C^2 and C^8 of purines come also from the β -carbon of serine. N^1 of purines and C^4 , C^5 , C^6 and N^1 of pyrimidines come from aspartate.

Glyoxylate

(a) Glycine is converted to glyoxylate either by transamination with glycine transaminase or by oxidative deamination with *glycine oxidase* (Fig. 12.3). Glyoxylate may be either transaminated to glycine or oxidized to oxalate.

(b) Catabolism of hydroxyproline also produces some glyoxylate along with pyruvate.

Nicotinamide

See pages 146-148.

Iodothyronines

These include thyroid hormones synthesized from tyrosine.

Labile methyl groups

Methyl groups which can be transferred from one molecule to another, are called labile methyl groups; e.g., the methyl groups of methionine, N^5 -methyl H_4 folate, methylcobalamin and betaine. Transmethylations or

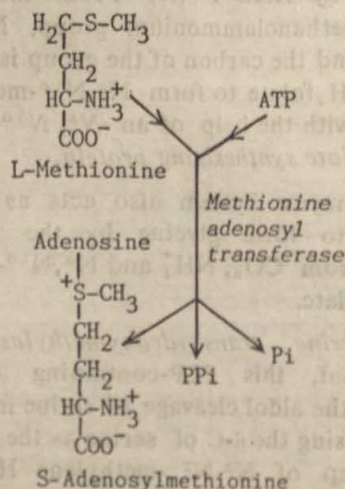


Fig. 12.32. Synthesis of S-adenosylmethionine.

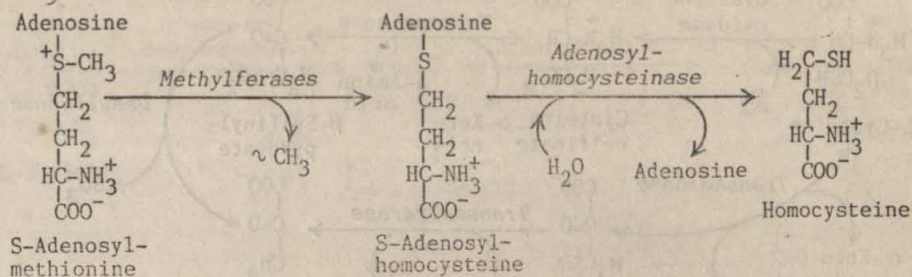


Fig. 12.33. Breakdown of methionine to homocysteine.

methyl group transfers are catalyzed by *methyltransferases*. For most of the trans-methylations in the body, the labile methyl group of methionine is utilized.

For transmethylation, methionine must first be changed to S-adenosylmethionine ('active' methionine) by *methionine adenosyl transferase* which transfers the adenosyl group of ATP to the sulfur atom of methionine, releasing PPi and Pi (Fig. 12.32). Addition of the adenosyl group endows the sulfur atom with a positive charge to make the methyl group more reactive with a higher transfer potential than N⁵-methyl H₄ folate. Probably a genetic deficiency of this enzyme produces *hypermethioninemia* characterized by high blood and urinary levels of methionine

and nervous symptoms like hypersomnolence and irritability.

On donating the methyl group to substrates, S-adenosylmethionine changes into S-adenosylhomocysteine which is hydrolyzed by *adenosylhomocysteinase* to adenosine and homocysteine (Fig. 12.33). The latter may be methylated back to methionine (see below). Some transmethylations are cited here.

(a) *Methylation of homocysteine to methionine*: The cytoplasmic enzyme *homocysteine methylferase* uses the methyl group of N⁵-methyl H₄ folate to methylate homocysteine to methionine; methylcobalamin is the mediator in this reaction (page 159, Fig. 7.26). *Betaine* may also be used as a methyl-donor for methylating homocysteine to methionine.

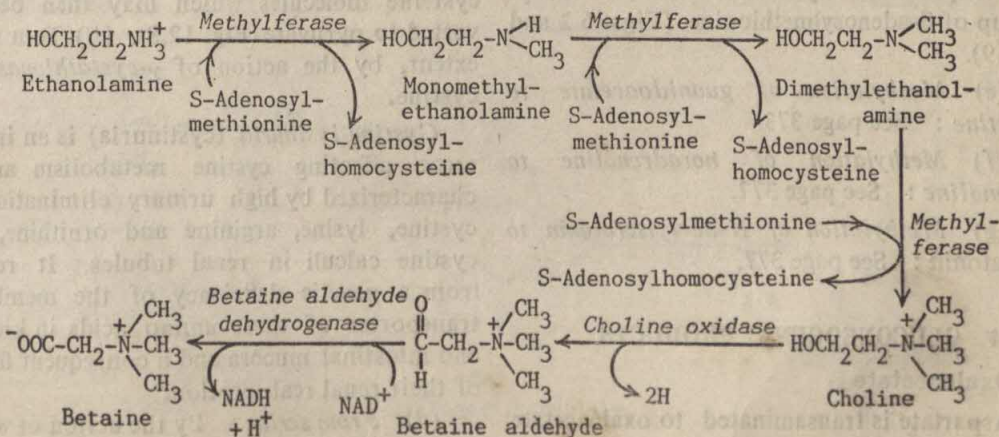


Fig. 12.34. Synthesis of choline and betaine.

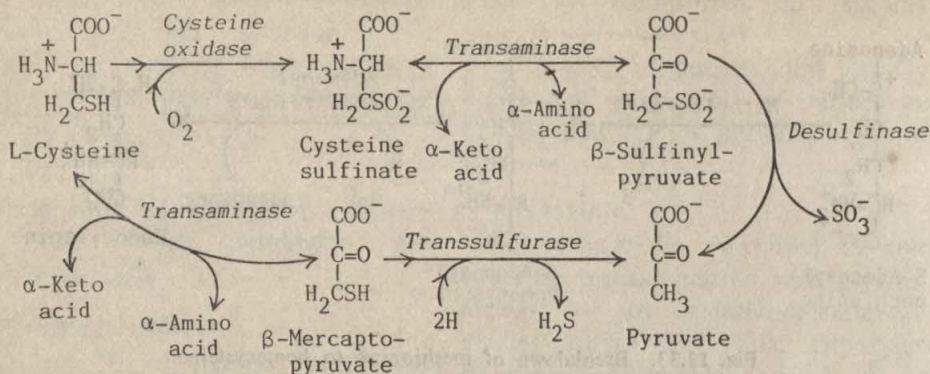


Fig. 12.35. Catabolism of cysteine to pyruvate.

(b) *Synthesis of choline and betaine:* Methylferases transfer methyl groups from three successive molecules of S-adenosylmethionine to ethanolamine in the liver to form choline (Fig. 12.34). The latter is either oxidized to betaine or used in synthesizing acetylcholine and phospholipids.

(c) *Methylation of phosphatidylethanolamine:* Methylferases transfer methyl groups from three successive molecules of S-adenosylmethionine to phosphatidylethanolamine, changing the latter to phosphatidylcholine.

(d) *Detoxications by methylation:* Histamine, nicotinamide, quinoline, thyroxine, estrogens and many other compounds are inactivated by methylation in the liver with the help of methylferases and the methyl group of S-adenosylmethionine (Figs. 6.2 and 12.19).

(e) *Methylation of guanidoacetate to creatine:* See page 373.

(f) *Methylation of noradrenaline to adrenaline:* See page 377.

(g) *Methylation of N-acetylserotonin to melatonin:* See page 377.

12.14 GLUCONEOGENIC PRODUCTS

Oxaloacetate

Aspartate is transaminated to oxaloacetate by aspartate transaminase (Fig. 12.1). As-

paragine forms oxaloacetate through aspartate.

Pyruvate

(a) *From alanine:* By transamination (Fig. 12.3).

(b) *From cysteine:* (i) Mainly through desulfhydration deamination by cysteine desulfhydrase (Fig. 12.8). (ii) By the action of a transsulfurase on β -mercaptopyruvate produced by transamination of cysteine (Fig. 12.35). (iii) Cysteine sulfinic acid, formed from cysteine by cysteine oxidase (Fig. 12.35), may be transaminated to β -sulfinylpyruvate which is desulfinated to pyruvate.

(c) *From cystine:* (i) Mainly through its reductive cleavage by cystine reductase to cysteine molecules which may then be converted to pyruvate (Fig. 12.8). (ii) To a small extent, by the action of γ -cystathionase on cystine.

Cystine-lysinuria (cystinuria) is an inborn error affecting cystine metabolism and is characterized by high urinary elimination of cystine, lysine, arginine and ornithine, and cystine calculi in renal tubules. It results from a genetic deficiency of the membrane transporter of these amino acids in kidneys and intestinal mucosa and a consequent failure of their renal reabsorption.

(d) *From serine:* By the action of serine dehydratase (Fig. 12.7).

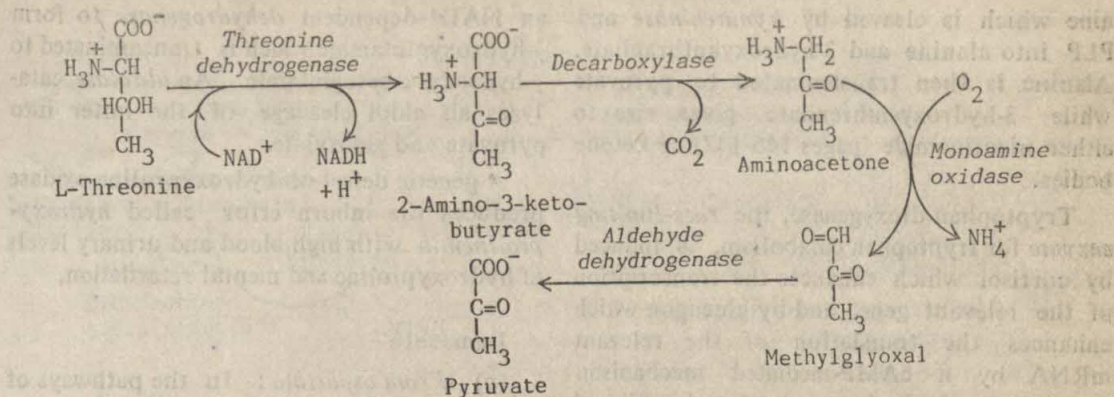


Fig. 12.36. Catabolism of threonine to pyruvate.

(e) *From glycine*: Through its conversion to serine by mitochondrial *serine transhydroxymethylase* (pages 383-384).

(f) *From threonine*: Threonine is oxidized by *threonine dehydrogenase* and NAD^+ to 2-amino-3-ketobutyrate which is then decarboxylated to aminacetone (Fig. 12.36). The latter is oxidatively deaminated by *monoamine oxidase* to methylglyoxal which is

subsequently oxidized by *aldehyde dehydrogenase* into pyruvate.

(g) *From tryptophan*: A heme enzyme called *tryptophan 2,3-dioxygenase* (tryptophan pyrrolase) oxidizes tryptophan to N-formylkynurenine which is deformylated by *kynurenine formylase* to kynurenine (Fig. 12.37). The latter is hydroxylated by NADPH and *kynurenine hydroxylase* to 3-hydroxykynurenine

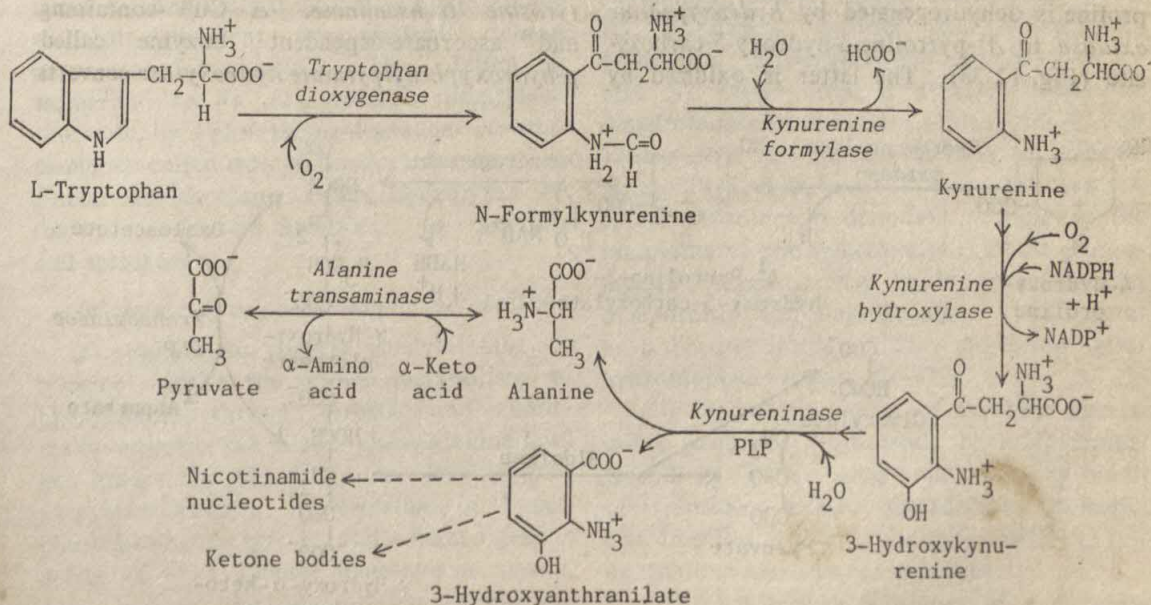


Fig. 12.37. Catabolism of tryptophan to pyruvate and 3-hydroxyanthranilate.

nine which is cleaved by *kynureninase* and PLP into alanine and 3-hydroxyanthranilate. Alanine is then transaminated to pyruvate while 3-hydroxyanthranilate gives rise to either nicotinamide (pages 146-147) or ketone bodies.

Tryptophan dioxygenase, the *rate-limiting enzyme* for tryptophan catabolism, is induced by cortisol which enhances the transcription of the relevant gene, and by glucagon which enhances the translation of the relevant mRNA by a cAMP-mediated mechanism. The enzyme is both repressed and inhibited by high levels of nicotinamide nucleotides, particularly NADPH. Its substrate tryptophan increases the activity of the enzyme by reducing its catabolism by proteolysis.

A genetic deficiency of kynurenine hydroxylase produces *kynureninuria* with urinary elimination of kynurenine. A genetic deficiency of kynureninase causes *xanthurenic aciduria* with urinary excretion of xanthurenic acid, an abnormal metabolite of tryptophan.

(h) *From hydroxyproline*: 4-Hydroxyproline is dehydrogenated by *hydroxyproline oxidase* to Δ^1 -pyrroline-3-hydroxy-5-carboxylate (Fig. 12.38). The latter is oxidized by

an NAD⁺-dependent *dehydrogenase* to form γ -hydroxyglutamate which is transaminated to γ -hydroxy- α -ketoglutarate. An *aldolase* catalyzes an aldol cleavage of the latter into pyruvate and glyoxylate.

A genetic defect of hydroxyproline oxidase produces the inborn error called *hydroxyprolinemia* with high blood and urinary levels of hydroxyproline and mental retardation.

Fumarate

(a) *From aspartate*: In the pathways of urea synthesis (Fig. 12.12) and purine synthesis (chapter 14).

(b) *From phenylalanine*: Phenylalanine is normally catabolized through tyrosine. Phenylalanine is hydroxylated to tyrosine with the help of *phenylalanine hydroxylase*, tetrahydrobiopterin and molecular O₂ (Fig. 12.39). Tetrahydrobiopterin gets simultaneously changed to dihydrobiopterin which is reduced back to tetrahydrobiopterin by NADPH and *dihydrobiopterin reductase*. Tyrosine is next transaminated to *p*-hydroxyphenylpyruvate by *tyrosine transaminase*. A Cu²⁺-containing and ascorbate-dependent enzyme called *p*-hydroxyphenylpyruvate hydroxylase converts

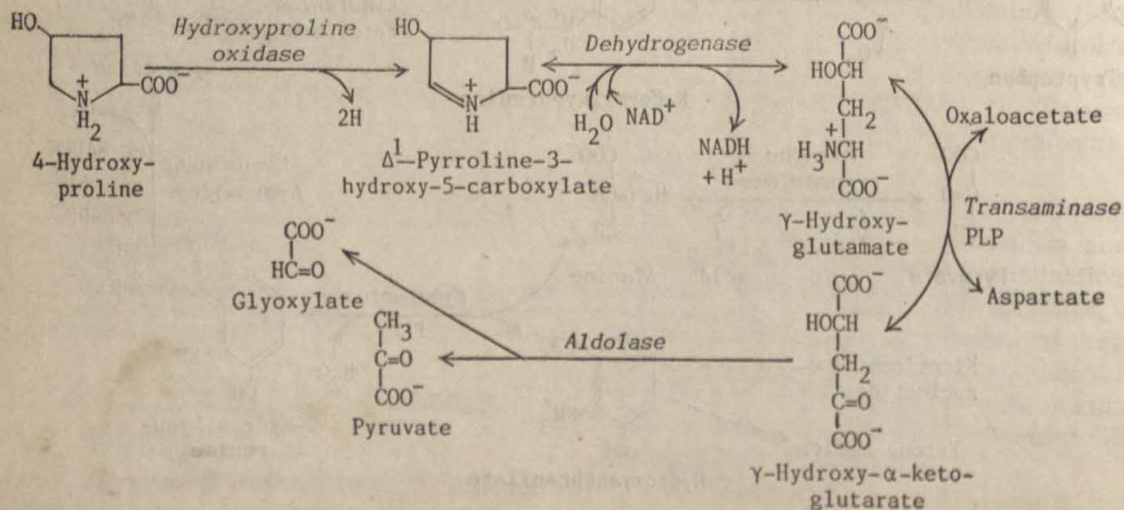


Fig. 12.38. Catabolism of hydroxyproline to pyruvate.

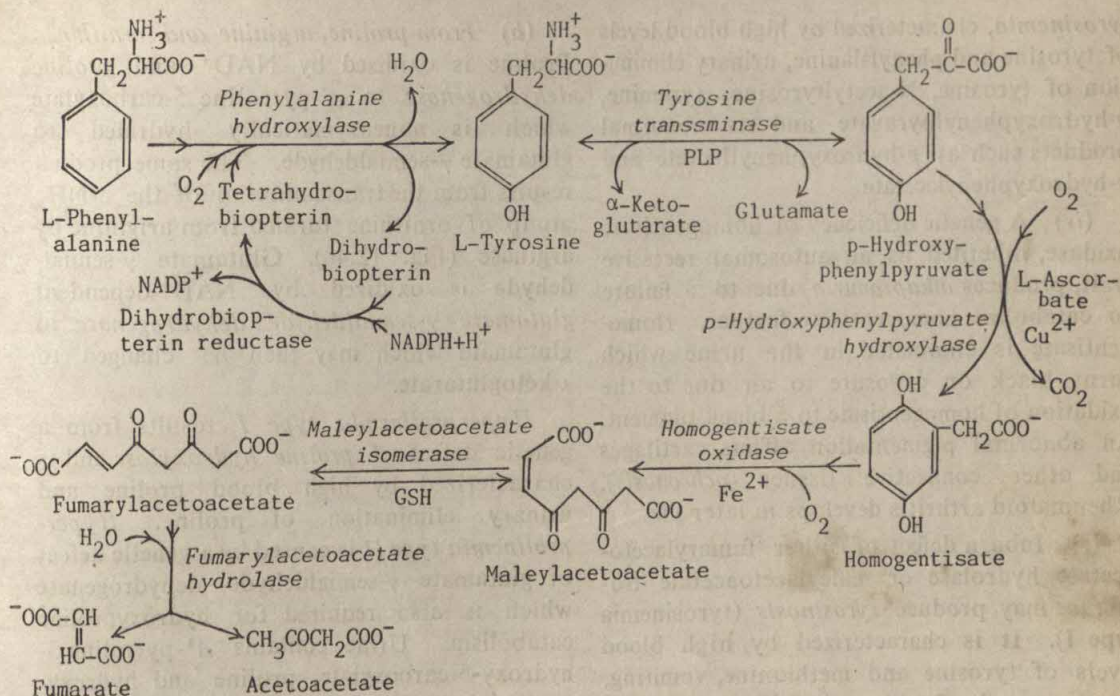


Fig. 12.39. Catabolism of phenylalanine to fumarate and acetoacetate.

p-hydroxyphenylpyruvate to homogentisate which is then oxidized by Fe^{2+} -containing and ascorbate-dependent *homogentisate oxidase* into maleylacetoacetate. The latter is isomerized to its *trans* isomer, fumarylacetoacetate, by a glutathione-dependent *cis-trans* isomerase called *maleylacetoacetate isomerase*. Fumarylacetoacetate is then hydrolyzed by *fumarylacetoacetate hydrolase* to fumarate and acetoacetate.

Inborn errors of phenylalanine metabolism :

(i) Failure to convert phenylalanine to tyrosine blocks the normal metabolism of phenylalanine through tyrosine and abnormally elevates the blood phenylalanine level but lowers the blood tyrosine level (*hyperphenylalaninemia*). *Phenylketonuria* (*hyperphenylalaninemia* type I) results from a genetic defect of phenylalanine hydroxylase; symptoms include high blood levels of Phe and its abnormal metabolites such as phenyl-

pyruvate and phenyllactate, their urinary elimination, severe mental retardation, poor IQ, psychoses, seizures and eczematous dermatitis. *Hyperphenylalaninemia* types II and III result from a genetic defect of dihydrobiopterin reductase while types IV and V arise from an inherited inability in synthesizing dihydrobiopterin; in these rare diseases, severe neurological disorders accompany the symptoms of phenylketonuria. These neurological symptoms may be due to deficiencies of serotonin and noradrenaline, synthesis of both these neurotransmitters requiring tetrahydrobiopterin (pages 376-377).

(ii) A genetic defect of tyrosine transaminase produces *tyrosinemia* type II, characterized by high blood and urinary levels of tyrosine, mental retardation, defective coordination, tendency for self-mutilation and ophthalmic and cutaneous lesions.

(iii) An inborn deficiency of *p*-hydroxyphenylpyruvate hydroxylase produces *neonatal*

tyrosinemia, characterized by high blood levels of tyrosine and phenylalanine, urinary elimination of tyrosine, N-acetyltyrosine, tyramine, *p*-hydroxyphenylpyruvate and its abnormal products such as *p*-hydroxyphenyllactate and *p*-hydroxyphenylacetate.

(iv) A genetic deficiency of homogentisate oxidase, inherited as an autosomal recessive trait, produces *alkaptonuria* due to a failure to catabolize homogentisate further. Homogentisate is eliminated in the urine which turns black on exposure to air due to the oxidation of homogentisate to a black pigment. An abnormal pigmentation affects cartilages and other connective tissues (*ochronosis*). Rheumatoid arthritis develops in later life.

(v) Inborn defect of either fumarylacetoacetate hydrolase or maleylacetoacetate isomerase may produce *tyrosinosis* (tyrosinemia type I). It is characterized by high blood levels of tyrosine and methionine, vomiting, diarrhoea and a fatal hepatic failure.

α -Ketoglutarate

(a) *From glutamate*: By (i) transamination (Fig. 12.1) and (ii) oxidative deamination catalyzed by glutamate dehydrogenase (Fig. 12.4).

(b) *From proline, arginine and ornithine*: Proline is oxidized by NAD^+ and *proline dehydrogenase* to Δ^1 -pyrroline 5-carboxylate which is nonenzymatically hydrated to glutamate γ -semialdehyde. The same product results from the transamination of the δ - NH_2 group of ornithine formed from arginine by arginase (Fig. 12.40). Glutamate γ -semialdehyde is oxidized by NAD^+ -dependent *glutamate γ -semialdehyde dehydrogenase* to glutamate which may then be changed to α -ketoglutarate.

Hyperprolinemia type I results from a genetic defect of *proline hydroxylase* and is characterized by high blood proline and urinary elimination of proline. *Hyperprolinemia type II* is caused by a genetic defect of glutamate γ -semialdehyde dehydrogenase which is also required for hydroxyproline catabolism. Urine contains Δ^1 -pyrroline-3-hydroxy-5-carboxylate, proline and hydroxyproline. Both diseases are inherited as autosomal recessive traits and frequently cause mental retardation.

(c) *From histidine*: Urocanate, produced from histidine by *histidase*, is changed to 4-imidazolone-5-propionate by an NAD^+ -dependent hepatic enzyme called *urocanase*.

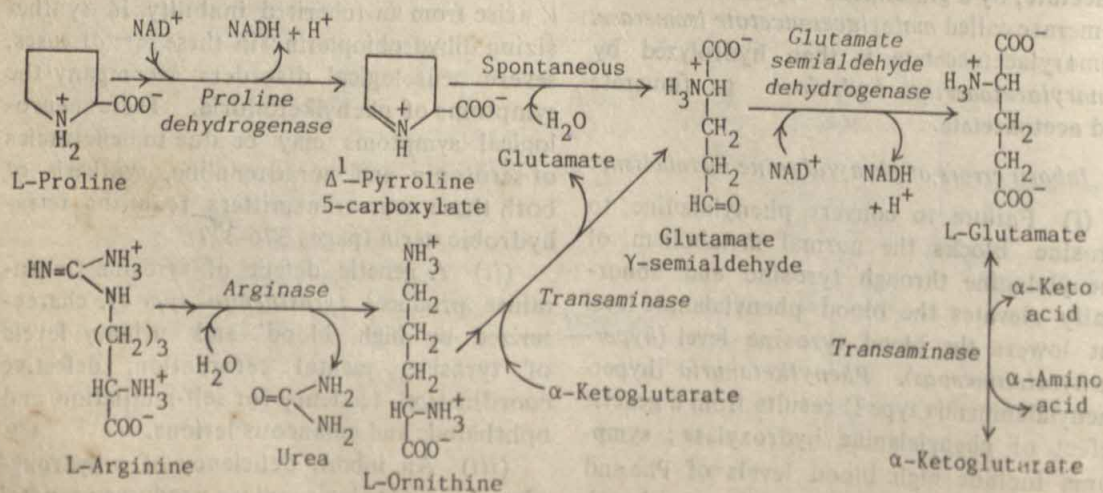
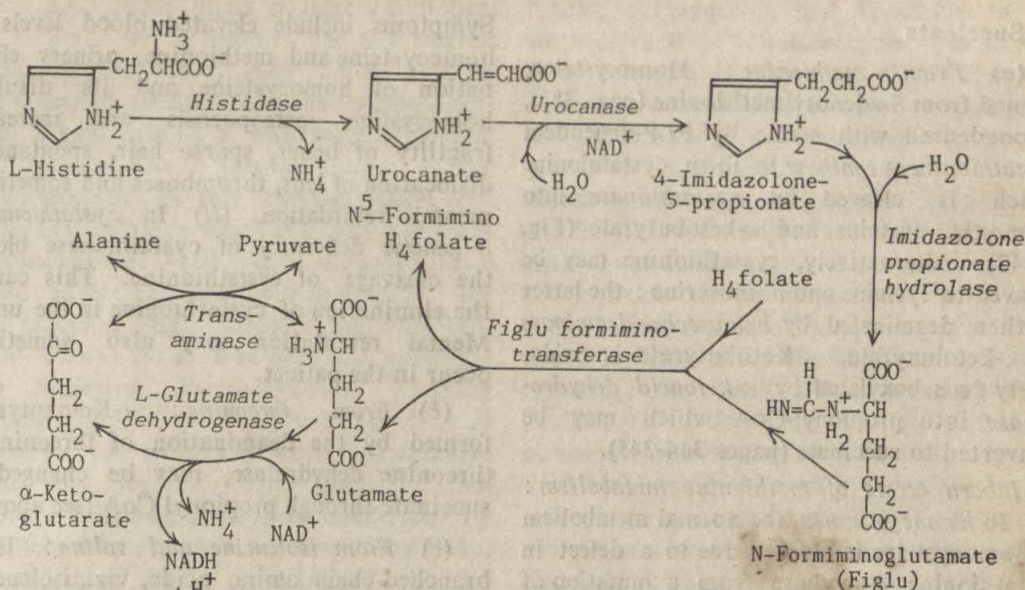


Fig. 12.40. Catabolism of proline, arginine and ornithine to α -ketoglutarate.

Fig. 12.41. Catabolism of histidine to α -ketoglutarate.

The imidazole ring is next cleaved by imidazolone propionate hydrolase to form N-formiminoglutamate (Figlu). Figlu formiminotransferase (glutamate formiminotransferase) transfers the formimino group of figlu to tetrahydrofolate, forming N^5 -formimino H_4folate and glutamate (Fig. 12.41). The latter may be changed to α -ketoglutarate. Genetic deficiency of histidase produces histidinemia (page 361).

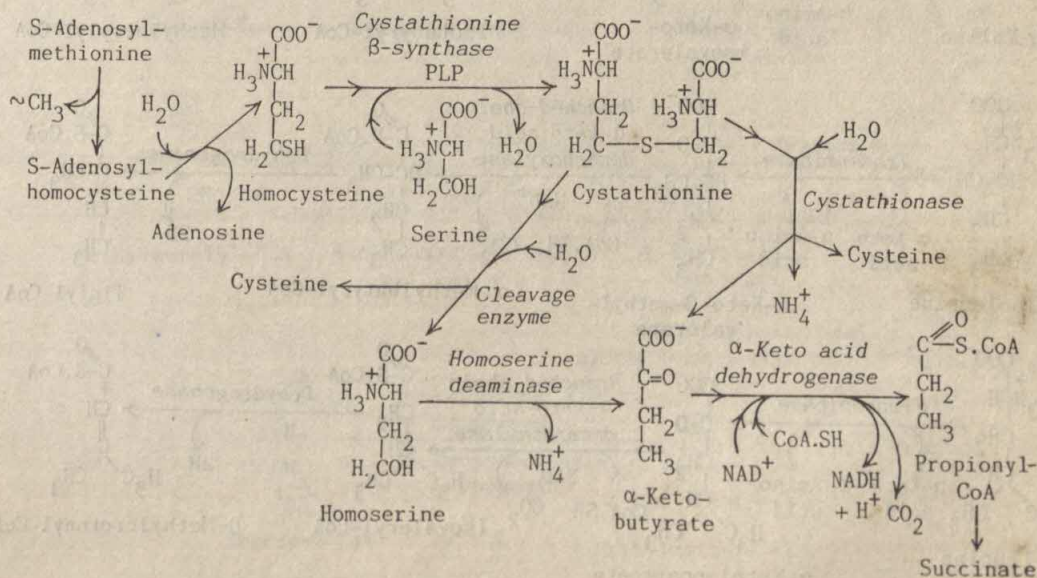


Fig. 12.42. Catabolism of methionine to succinate.

Succinate

(a) *From methionine*: Homocysteine, formed from S-adenosylmethionine (page 385), is condensed with serine by PLP-dependent *cystathionine β -synthase* to form cystathionine which is cleaved by *cystathionase* into ammonia, cysteine and α -ketobutyrate (Fig. 12.42). Alternatively, cystathionine may be cleaved to cysteine and homoserine; the latter is then deaminated by *homoserine deaminase* to α -ketobutyrate. α -Ketobutyrate is oxidatively decarboxylated by α -ketoacid dehydrogenase into propionyl-CoA which may be converted to succinate (pages 344-345).

Inborn errors of methionine metabolism:

(i) In *homocystinuria*, the normal metabolism of homocysteine is blocked due to a defect in cystathionine β -synthase from a mutation of either its catalytic site or its PLP-binding site.

Symptoms include elevated blood levels of homocysteine and methionine, urinary elimination of homocysteine and its disulfide homocystine, osteoporosis and increased fragility of bones, sparse hair, spontaneous dislocation of lens, thromboses and sometimes mental retardation. (ii) In *cystathionuria*, a genetic deficiency of cystathionase blocks the cleavage of cystathionine. This causes the elimination of cystathionine in the urine. Mental retardation may also sometimes occur in the patient.

(b) *From threonine*: α -Ketobutyrate, formed by the deamination of threonine by threonine dehydratase, may be changed to succinate through propionyl-CoA (see above).

(c) *From isoleucine and valine*: Three branched-chain amino acids, viz., isoleucine, valine and leucine, undergo the same reactions

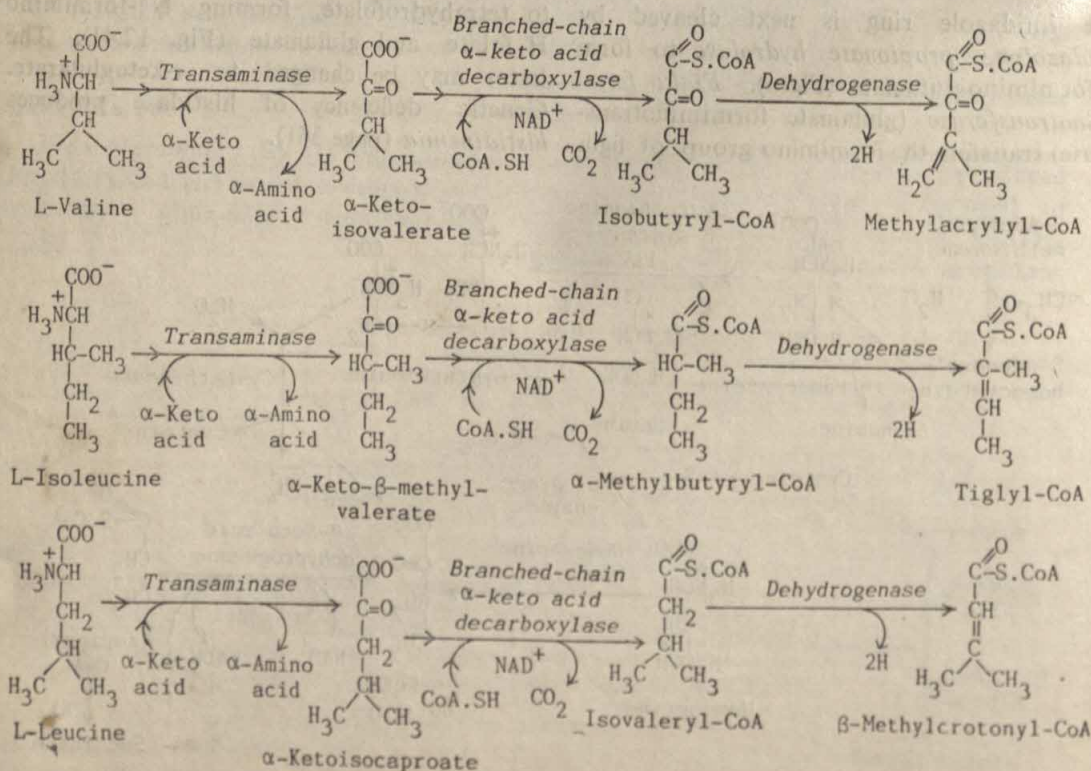


Fig. 12.43. The first three analogous reactions in the catabolism of branched-chain amino acids.

in the first three steps of their catabolism (Fig. 12.43). (i) In muscles and to a smaller extent in the liver, each amino acid is transaminated to the corresponding branched-chain α -keto acid by a *branched-chain amino acid transaminase*; pyruvate serves as the amino-acceptor in this transamination and changes into alanine which is largely used for gluconeogenesis. (ii) The branched-chain α -keto acids are then transported to the liver where a single mitochondrial *branched-chain α -keto acid decarboxylase* catalyzes their oxidative decarboxylation, forming α -methylbutyryl-CoA, isobutyryl-CoA and isovaleryl-CoA from respectively isoleucine, valine and leucine. This enzyme, like pyruvate dehydrogenase, gets inactivated by a protein kinase-catalyzed phosphorylation, is reactivated by a protein phosphatase-catalyzed dephosphorylation, requires TPP,

NAD⁺, coenzyme A and lipoamide as coenzymes, and is a multienzyme system with three enzymic components, viz., branched-chain α -keto acid decarboxylase, transacylase and dihydrolipoyl dehydrogenase. (iii) The acyl-CoA molecules, produced in this reaction, are then dehydrogenated to corresponding Δ^2 -trans-enoyl-CoA molecules, viz., tiglyl-CoA, methylacrylyl-CoA and β -methylcrotonyl-CoA from respectively isoleucine, valine and leucine. Human hepatic mitochondria have two dehydrogenases for this reaction, one acting on isovaleryl-CoA (*isovaleryl-CoA dehydrogenase*) and the other on the two other acyl-CoA molecules.

After these three common steps, the enoyl-CoA from each amino acid follows specific subsequent reactions. Tiglyl-CoA from isoleucine is hydrated by a *crotonase* (enoyl-CoA hydratase) to α -methyl- β -hydroxybutyryl-

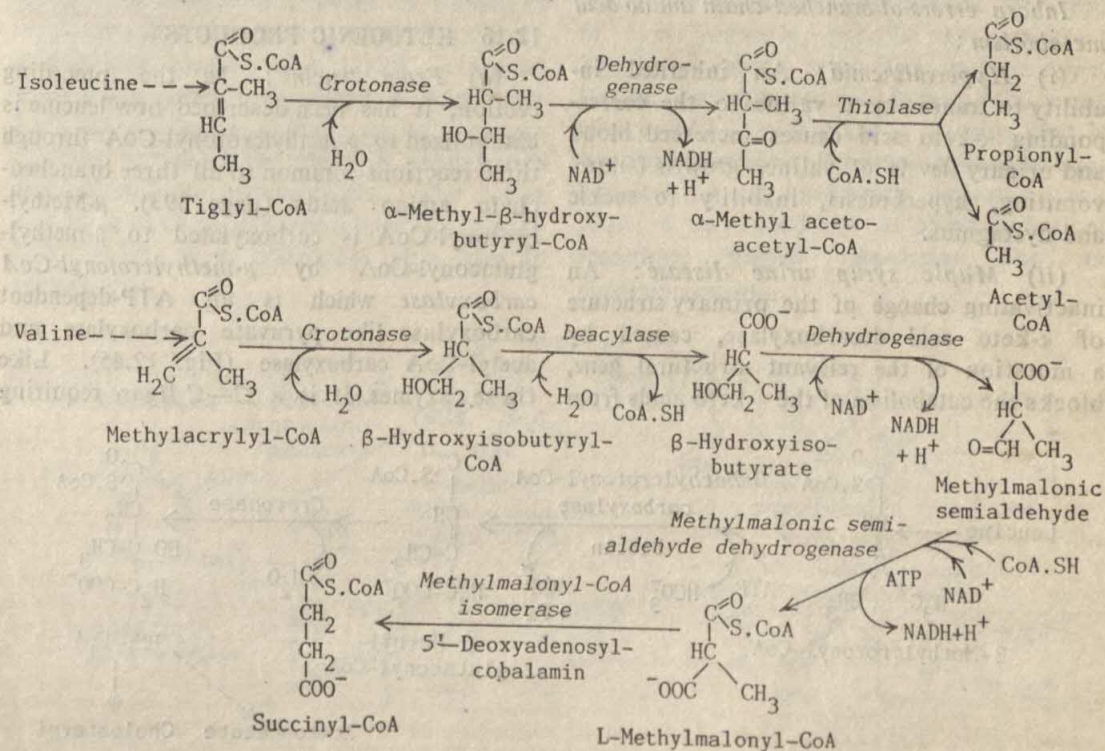


Fig. 12.44. Catabolism of tiglyl-CoA from isoleucine and methylacrylyl-CoA from valine

CoA; the latter is oxidized by an NAD^+ -dependent *dehydrogenase* to α -methylacetyl-CoA which is thiolized by coenzyme A into acetyl-CoA and propionyl-CoA, the latter subsequently changing into succinate (Fig. 12.44). On the other hand, methylacrylyl-CoA from valine is hydrated by a *crotonase* into β -hydroxyisobutyryl-CoA which is deacylated to β -hydroxyisobutyrate by a specific *deacylase* (Fig. 12.44); the product is oxidized by NAD^+ -dependent β -hydroxyisobutyrate *dehydrogenase* to methylmalonate semialdehyde which is oxidized and thioesterified by *methylmalonic semialdehyde dehydrogenase*, NAD^+ and coenzyme A to form L-methylmalonyl-CoA. The latter is isomerized to succinyl-CoA by *methylmalonyl-CoA isomerase* and 5'-deoxyadenosyl cobalamin. The fate of β -methylcrotonyl-CoA, the metabolite of leucine, is described in the next section.

Inborn errors of branched-chain amino acid metabolism:

(i) *Hypervalinemia*: An inherited inability to transaminate valine to the corresponding α -keto acid causes increased blood and urinary levels of valine, growth failure, vomiting, hyperkinesia, inability to suckle and nystagmus.

(ii) *Maple syrup urine disease*: An inactivating change of the primary structure of α -keto acid decarboxylase, caused by a mutation of the relevant structural gene, blocks the catabolism of the α -keto acids from

all three branched-chain amino acids. This raises the blood levels of valine, leucine and isoleucine; these amino acids and their α -keto acids are eliminated in the urine which smells characteristically like burnt sugar or maple syrup. Other symptoms include depressions in α -ketoglutarate dehydrogenase and glutamate dehydrogenase activities, brain damage, mental retardation, vomiting, poor muscle tone, abnormal movements, respiratory failure, lethargy and convulsive seizures. The affected infant dies unless treated early.

(iii) *Isovaleric acidemia*: A genetic defect of isovaleryl-CoA dehydrogenase blocks the catabolism of isovaleryl-CoA, an intermediate in leucine metabolism. This results in elevated plasma, sweat and urinary levels of isovalerate, mental retardation, lethargy, acidosis, vomiting, characteristic 'cheesy' odour of breath and coma.

12.15 KETOGENIC PRODUCTS

(a) *From leucine*: In the preceding section, it has been described how leucine is catabolized to β -methylcrotonyl-CoA through three reactions common to all three branched-chain amino acids (page 393). β -Methylcrotonyl-CoA is carboxylated to β -methylglutaconyl-CoA by β -methylcrotonyl-CoA carboxylase which is an ATP-dependent carboxylase like pyruvate carboxylase and acetyl-CoA carboxylase (Fig. 12.45). Like those enzymes, it is a C—C ligase requiring

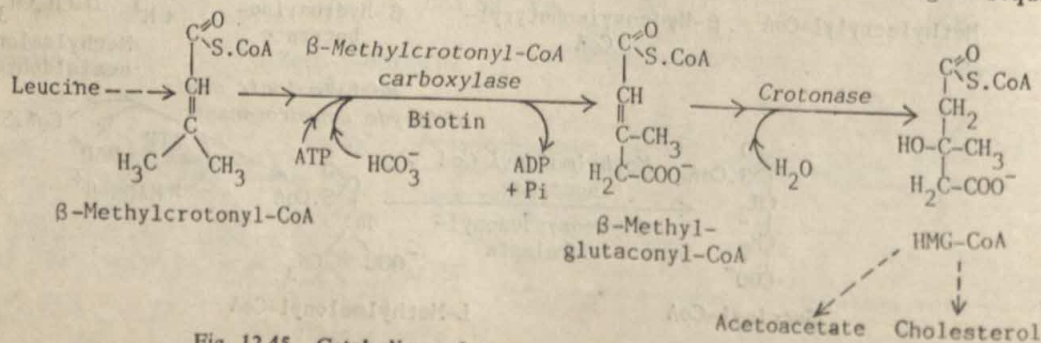


Fig. 12.45. Catabolism of β -methylcrotonyl-CoA from leucine.

biotin as the prosthetic group and forms carboxybiotin as an intermediate (page 155). β -Methylglutaconyl CoA is next hydrated by *crotonase* to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). HMG-CoA may give rise to acetoacetate or to cholesterol.

(b) *From phenylalanine and tyrosine*: It has been described on pages 388-389 how these amino acids are catabolized into fumarate as well as acetoacetate.

(c) *From isoleucine*: Isoleucine is catabolized to succinate as well as acetyl-CoA (pages 393-394). The latter may give rise to acetoacetate.

(d) *From tryptophan*: It has been described on pages 387-388 how tryptophan is catabolized to pyruvate (through alanine) and 3-hydroxyanthranilate. The latter is changed to α -amino- β -carboxymuconic semialdehyde through an oxidative cleavage of its ring by a ferroprotein oxygenase called *3-hydroxyanthranilate oxidase* (Fig. 12.46). α -Amino- β -carboxymuconic semialdehyde is decarboxylated by *picolinate carboxylase* to α -aminomuconic semialdehyde which is deaminated and reduced to α -ketoadipate. The latter may be oxidatively decarboxylated to glutaryl-CoA which may ultimately give rise to acetoacetate.

(e) *From lysine*: Lysine cannot be transaminated. Instead, it is condensed with α -ketoglutarate to form a Schiff base complex which is reduced to saccharopine by NADH and a *lysine-forming saccharopine dehydrogenase* (Fig. 12.47). Saccharopine is next oxidized by a *glutamate-forming saccharopine dehydrogenase*. The product is cleaved by water to glutamate and α -aminoadipate semialdehyde. In this roundabout way, lysine loses its ϵ -amino group. α -Aminoadipate semialdehyde is oxidized by an NAD⁺-dependent dehydrogenase to α -aminoadipate which loses its α -amino group by transamination to form α -ketoadipate. The latter is changed to glutaryl-CoA (Fig. 12.46) which gives rise to acetoacetate.

Inborn defects of the enzymes for converting lysine to saccharopine produce two types of *hyperlysinemia*, periodic and persistent. The blood lysine level rises in both types. Periodic hyperlysinemia, however, occurs only after protein ingestion and is accompanied by hyperammonemia. Persistent hyperlysinemia, inherited as an autosomal recessive trait, shows a continued rise in blood lysine and sometimes mental retardation, but no hyperammonemia.

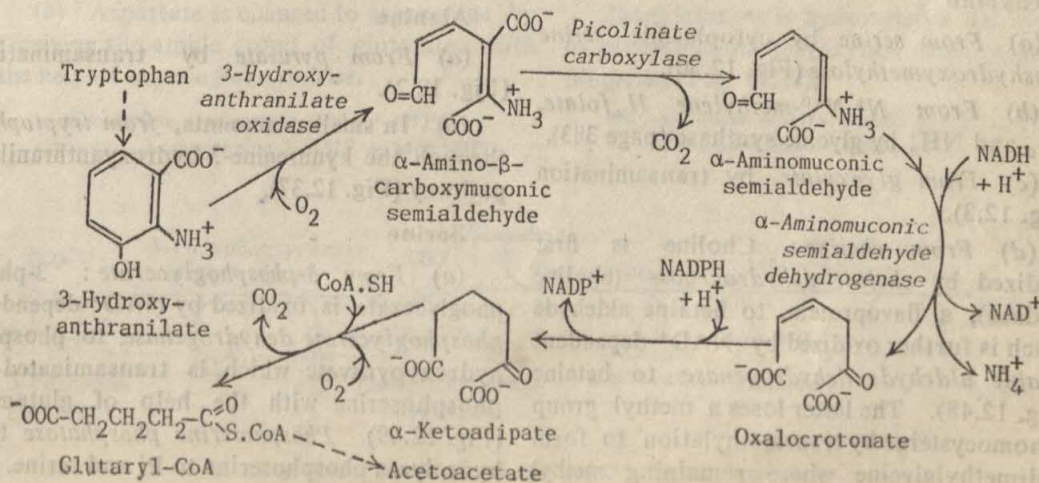


Fig. 12.46. Catabolism of 3-hydroxyanthranilate from tryptophan.

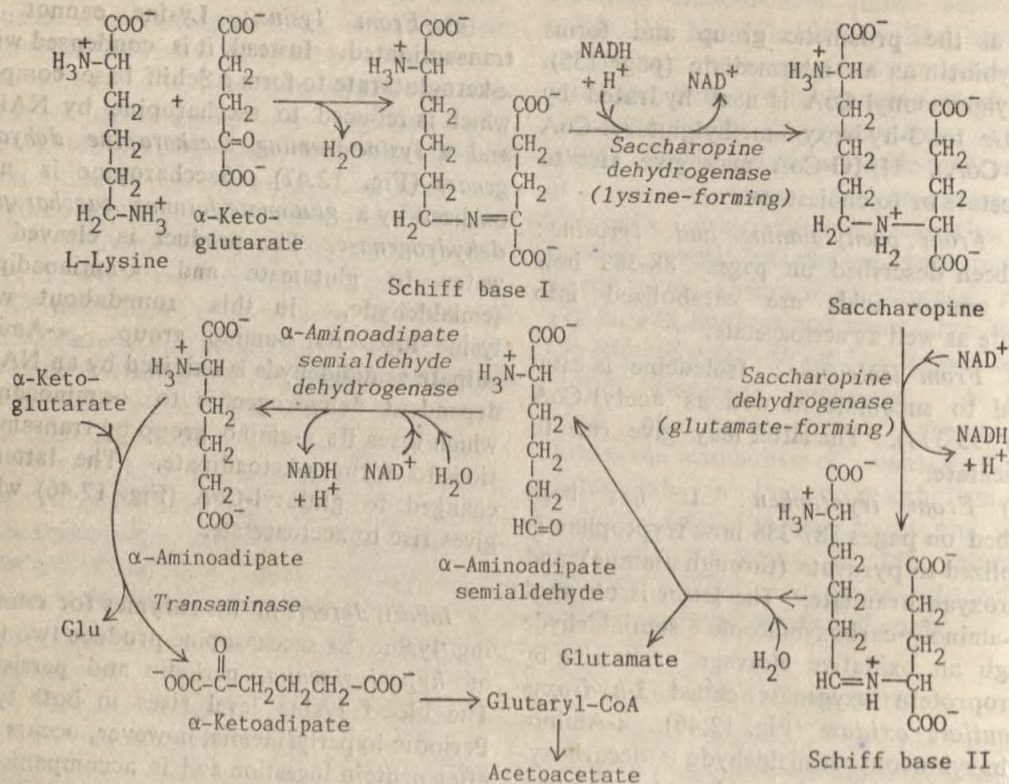


Fig. 12.47. Catabolism of lysine.

12.16 MAMMALIAN BIOSYNTHESIS OF AMINO ACIDS

Glycine

(a) From serine by cytoplasmic serine transhydroxymethylase (Fig. 12.30).

(b) From N⁵,N¹⁰-methylene H₄folate, CO₂ and NH₄⁺ by glycine synthase (page 383).

(c) From glyoxylate by transamination (Fig. 12.3).

(d) From choline: Choline is first oxidized by choline dehydrogenase (choline oxidase), a flavoprotein, to betaine aldehyde which is further oxidized by NAD⁺-dependent betaine aldehyde dehydrogenase to betaine (Fig. 12.48). The latter loses a methyl group to homocysteine by transmethylation to form N-dimethylglycine whose remaining methyl

groups are removed as formaldehyde by successive oxidations by dimethylglycine oxidase and sarcosine oxidase to give glycine.

Alanine

(a) From pyruvate by transamination (Fig. 12.2).

(b) In smaller amounts, from tryptophan through the kynurenine-3-hydroxyanthranilate pathway (Fig. 12.37).

Serine

(a) From 3-phosphoglycerate: 3-phosphoglycerate is oxidized by NAD⁺-dependent phosphoglycerate dehydrogenase to phosphohydroxypyruvate which is transaminated to phosphoserine with the help of glutamate (Fig. 12.49). Phosphoserine phosphatase then hydrolyzes phosphoserine to Pi and serine.

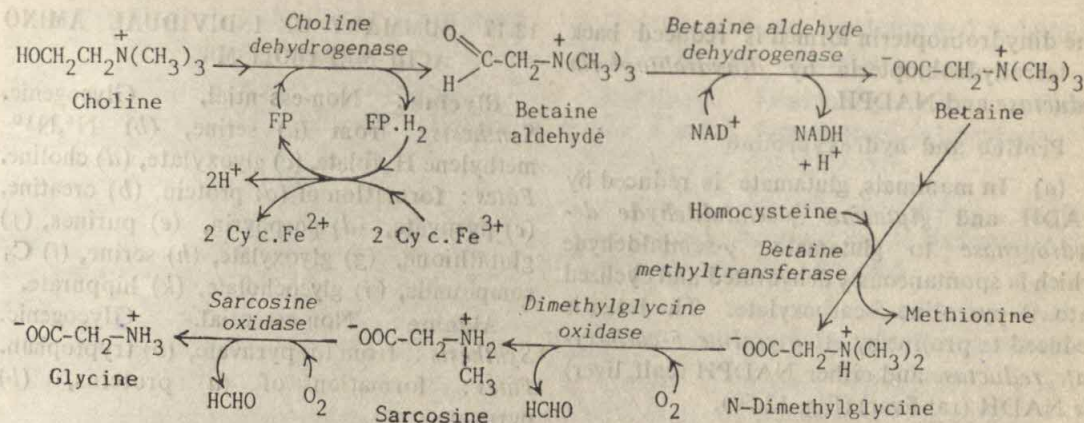


Fig. 12.48. Synthesis of glycine from choline.

(b) *From glycine with the help of mitochondrial serine transhydroxymethylase and N⁵,N¹⁰-methylene H₂folate* (pages 383-384).

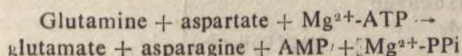
Cysteine

From methionine and serine: Homocysteine, formed from S-adenosylmethionine, is condensed with serine by PLP-dependent *cystathionine β -synthase* to form cystathionine which is cleaved into cysteine and either homoserine or α -ketobutyrate (Fig. 12.42).

Aspartate and asparagine

(a) Aspartate is formed by transamination of oxaloacetate (Fig. 12.1).

(b) Aspartate is changed to asparagine by receiving the amide group of glutamine with the help of *asparagine synthase*.

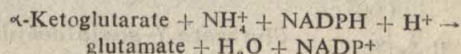


The PPI formed is immediately hydrolyzed to two Pi molecules by a *pyrophosphatase* to drive the reaction towards asparagine formation.

Glutamate

(a) By transamination of α -ketoglutarate (Fig. 12.1).

(b) By reductive amination of α -ketoglutarate by NADPH and the cytoplasmic isozyme of L-glutamate dehydrogenase (page 360).



Tyrosine

Phenylalanine is hydroxylated to tyrosine by *phenylalanine hydroxylase* and tetrahydrobiopterin (Fig. 12.39).

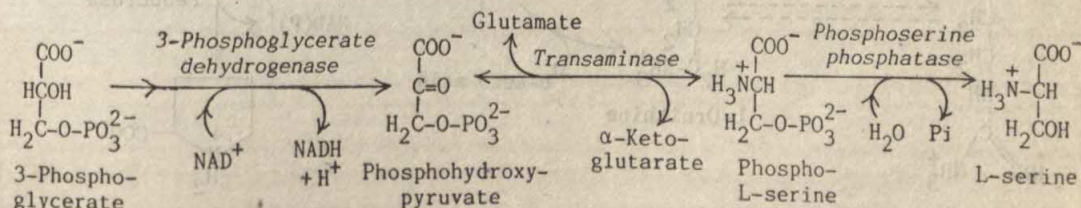
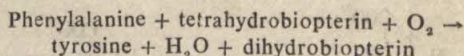


Fig. 12.49. Synthesis of serine from 3-phosphoglycerate.

The dihydrobiopterin formed is reduced back to tetrahydrobiopterin by *dihydrobiopterin reductase* and NADPH.

Proline and hydroxyproline

(a) In mammals, glutamate is reduced by NADH and *glutamate γ -semialdehyde dehydrogenase* to glutamate γ -semialdehyde which is spontaneously dehydrated and cyclized into Δ^1 -pyrroline 5-carboxylate. The latter is reduced to proline by *Δ^1 -pyrroline 5-carboxylate reductase* and either NADPH (calf liver) or NADH (rat liver) (Fig. 12.50).

(b) Microsomal *prolyl 4-hydroxylase* hydroxylates proline residues in collagen to 4-hydroxyproline residues, utilizing L-ascorbate and α -ketoglutarate as cofactors (page 138).

Arginine and ornithine

(a) Ornithine is formed by the transamination of γ -carboxyglutamate produced from glutamate (Fig. 12.50).

(b) Arginine is synthesized from ornithine in the *arginine-urea pathway* for urea synthesis (pages 368-369).

12.17 SUMMARY OF INDIVIDUAL AMINO ACID METABOLISMS

Glycine: Non-essential. Glycogenic.

Synthesis: from (a) serine, (b) N^5, N^{10} -methylene H_4 folate, (c) glyoxylate, (d) choline.
Fates: formation of (a) protein, (b) creatine, (c) pyruvate, (d) porphyrin, (e) purines, (f) glutathione, (g) glyoxylate, (h) serine, (i) C_1 compounds, (j) glycocholate, (k) hippurate.

Alanine: Non-essential. Glycogenic.

Synthesis: from (a) pyruvate, (b) tryptophan.
Fates: formation of (a) proteins, (b) pyruvate.

Serine: Non-essential. Glycogenic. Syn-

thesis: from (a) 3-phosphoglycerate, (b) glycine. **Fates:** formation of (a) proteins, (b) pyruvate, (c) glycine, (d) sphingosine, (e) C_1 compounds, (f) cysteine.

Cysteine: Non-essential. Glycogenic.

Synthesis: from methionine and serine. **Fates:** formation of (a) proteins, (b) cystine, (c) pyruvate, (d) taurine, (e) glutathione.

Threonine: Essential. Glycogenic. **Fates:** formation of (a) proteins, (b) pyruvate, (c) succinate.

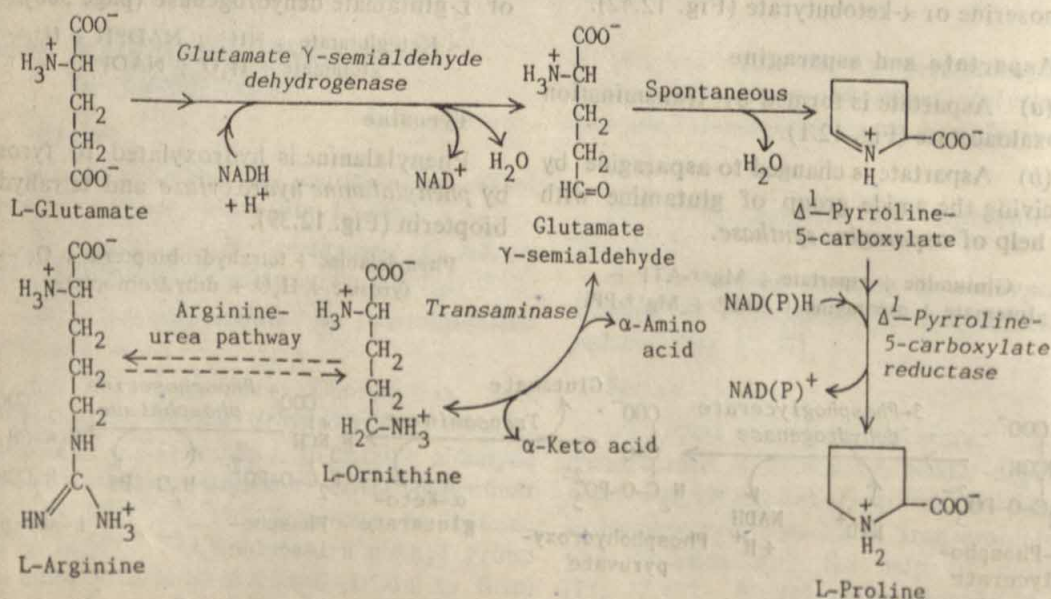


Fig. 12.50. Synthesis of proline, ornithine and arginine.

Aspartate : Non-essential. Glycogenic. *Synthesis :* from oxaloacetate. *Fates :* formation of (a) proteins, (b) asparagine, (c) oxaloacetate, (d) urea and arginine, (e) pyrimidines, (f) purines, (g) fumarate.

Glutamate : Non-essential. Glycogenic. *Synthesis :* (a) transamination of α -ketoglutarate, (b) L-glutamate dehydrogenase action on α -ketoglutarate. *Fates :* formation of (a) proteins, (b) α -ketoglutarate, (c) glutamine, (d) glutathione, (e) γ -aminobutyrate, (f) proline.

Methionine : Essential. Glycogenic. *Fates :* formation of (a) proteins, (b) S-adenosylmethionine as a source of labile methyl groups, (c) choline, epinephrine, melatonin and creatine by transmethylation, (d) cysteine, (e) succinate, (f) polyamines.

Phenylalanine and tyrosine : Essential and non-essential, respectively. Glycogenic-ketogenic. *Synthesis of tyrosine :* from phenylalanine. *Fates :* formation of (a) proteins, (b) catecholamines, (c) melanins, (d) iodothyronines, (e) fumarate, (f) acetoacetate.

Tryptophan : Essential Glycogenic-ketogenic. *Fates :* formation of (a) proteins,

(b) nicotinamide, (c) serotonin and melatonin, (d) pyruvate, (e) acetoacetate.

Histidine : Semi-indispensable. Glycogenic. *Fates :* formation of (a) proteins, (b) histamine, (c) α -ketoglutarate.

Lysine : Essential. Ketogenic. *Fates :* formation of (a) proteins, (b) hydroxylysine, (c) acetoacetate, (d) carnitine.

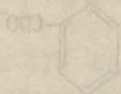
Leucine : Essential. Ketogenic. *Fates :* formation of (a) proteins, (b) acetoacetate.

Valine : Essential. Glycogenic. *Fates :* formation of (a) proteins, (b) succinate.

Isoleucine : Essential. Glycogenic-ketogenic. *Fates :* formation of (a) proteins, (b) succinate, (c) acetate.

Proline : Non-essential. Glycogenic. *Synthesis :* from glutamate. *Fates :* formation of (a) proteins, (b) hydroxyproline, (c) α -ketoglutarate.

Arginine and ornithine : Glycogenic. *Synthesis :* (a) ornithine from glutamate. (b) arginine from ornithine. *Fates :* formation of (a) urea, (b) α -ketoglutarate, (c) creatine (for arginine only), (d) proteins, (for arginine only), (e) polyamines (for ornithine only).



Phenylalanine

Fig. 11.1. Oxidation of phenylalanine and
tyrosine (from amino acid metabolism, 2nd edn.)

for oxidation occur in mitochondria and
fatty acids collected or synthesized in the
cytoplasm must be "activated" as well as
transferred into mitochondria for oxidation.

Activation of fatty acids

Before the transfer into mitochondria fatty
acids need to be changed by fatty acid
thioesterase (acyl-CoA synthetase) into active
intermediates called acyl-CoA or fatty acid
CoA thioester. Thioesterase C-2 (HMG

CoA synthetase) is the enzyme
of the cell with which fatty acids are
activated. The reaction is reversible and
the reaction is coupled with the hydrolysis
of ATP to ADP and inorganic phosphate.
The reaction is coupled with the hydrolysis
of ATP to ADP and inorganic phosphate.

Knop found phenylalanine in the urine
of dogs fed with phenylalanyl glycine.
Fatty acids like phenylalanine, but when fed
with phenylalanyl glycine, fatty acids
like phenylalanine does excrete benzoic
acid (Fig. 11.1). So, Knop pro-
posed that a fatty acid would be activated
mixed at the reaction (1) releasing an
active acid (2) at each step. This original
oxidation theory was subsequently modified
by Fischer, Durr, Lyon, Berg and others.
It has been demonstrated that the enzyme

13. METABOLISM OF LIPIDS

Lipids are partly obtained from foods and partly derived from carbohydrates. Fats are stored in adipocytes as highly concentrated sources of energy and are oxidized for energy production. Subcutaneous fats serve in thermoinsulating the body against heat loss. Fat deposits function also as shock-absorbing cushions. Phosphoglycerides, sphingolipids and cholesterol are essential structural components. Vitamin D, bile salts and steroid hormones are synthesized from cholesterol. Prostaglandins and related compounds, synthesized from fatty acids, possess many biological actions.

13.1 BETA-OXIDATION

Fatty acids are oxidized aerobically by β -oxidation in the mitochondrial matrix of liver, muscles, heart, renal cortex and brown adipose tissue. This serves much of the energy-need during starvation, carbohydrate deprivation, cold thermogenesis and sustained muscular work such as marathon runs, prolonged flights of birds and insects, and prolonged swims of whales and migratory fishes.

Knoop found phenylacetate in the urine of dogs fed with phenyl-substituted even-C fatty acids like phenylbutyrate; but when fed with phenyl-substituted odd-C fatty acids like phenylpropionate, dogs excreted benzoate (as hippurate) (Fig. 13.1). So, Knoop proposed that a fatty acid would be stepwise oxidized at its β -carbon (C^2), releasing an acetic acid (C_2) at each step. This original β -oxidation theory was subsequently modified by Embden, Dakin, Lynen, Berg and others. It has been demonstrated that the enzymes

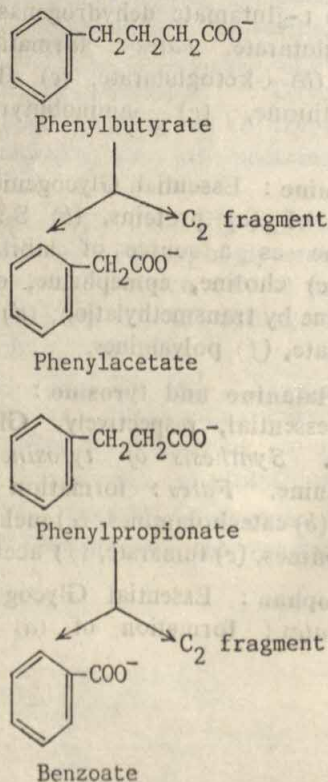


Fig. 13.1. Formation of phenylacetate and benzoate from phenyl-substituted fatty acids.

for β -oxidation occur in mitochondria and fatty acids, collected or synthesized in the cytoplasm, must be "activated" as well as transferred into mitochondria for β -oxidation.

Activation of fatty acids

Before the transfer into mitochondria, fatty acids need to be changed by fatty acid thiokinases (acyl-CoA synthases) into active intermediates called acyl-CoA or fatty acid-CoA thioesters. Thiokinases are C-S ligases

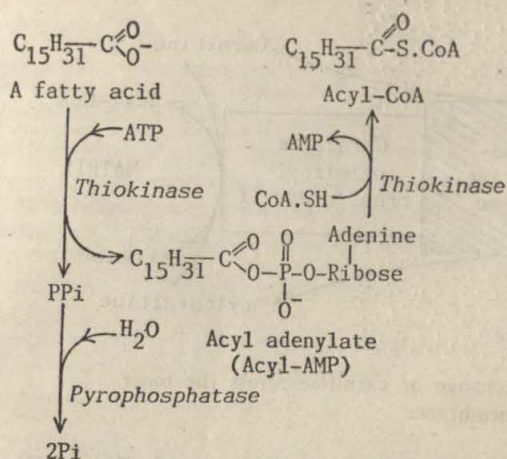
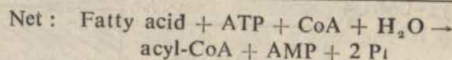
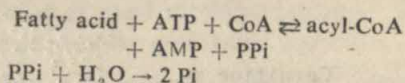


Fig. 13.2. Action of fatty acid thiokinase.

occurring on the outer mitochondrial membrane and in the mitochondrial matrix. They possess chain-length specificity and thioesterify fatty acids of specific chain-lengths; e.g., long-chain fatty acid thiokinases for palmitate (C_{16}) and stearate (C_{18}), medium-chain fatty acid thiokinases for C_4 - C_{12} acids like octanoic acid, and acetate thiokinase (acetyl-CoA synthase) for acetate and propionate.

A thiokinase first utilizes ATP for adenylating the fatty acid into an enzyme-bound acyl adenylate with the release of pyrophosphate. The acyl adenylate is a carboxylic-phosphoric anhydride in which the COOH group of the fatty acid remains linked to the phosphate group of AMP. Coenzyme A next reacts with acyl adenylate; this results in the release of acyl-CoA and AMP from the enzyme (Fig. 13.2). Though freely reversible, the thiokinase reaction is almost unidirectional in the body because the PPi is immediately hydrolyzed to two Pi molecules by pyrophosphatase. In effect, thiokinases form a thioester linkage between the carboxyl-C of a fatty acid and the thiol-S of coenzyme A at the cost of 2 high-energy bonds of ATP.



Lower fatty acids like acetic and propionic acids first diffuse freely into mitochondria and then get thioesterified to respective acyl-CoA molecules by thiokinases of mitochondrial matrix.

Carnitine shuttle

Long-chain acyl groups are carried across the inner mitochondrial membrane by a mobile carrier called *carnitine* (β -hydroxy- γ -trimethylammonium butyrate). The latter is synthesized in liver and kidneys from lysine and methionine (pages 380-381). *Carnitine palmitoyltransferase I*, located on the cytoplasmic surface of the inner membrane, transfers the acyl group from cytoplasmic acyl-CoA to carnitine, forming acylcarnitine and free coenzyme A (Fig. 13.3). An inner membrane protein, called *carnitine-acylcarnitine translocase*, then transports acylcarnitine across the membrane into the mitochondria in exchange of carnitine transported out from the mitochondria (Fig. 13.4). Reaching the mitochondrial matrix, acylcarnitine donates its acyl group to coenzyme A under the action

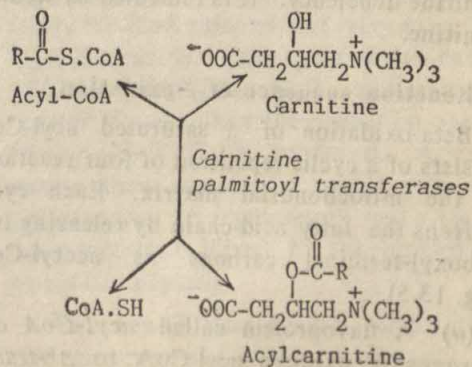


Fig. 13.3. Formation of acylcarnitine.

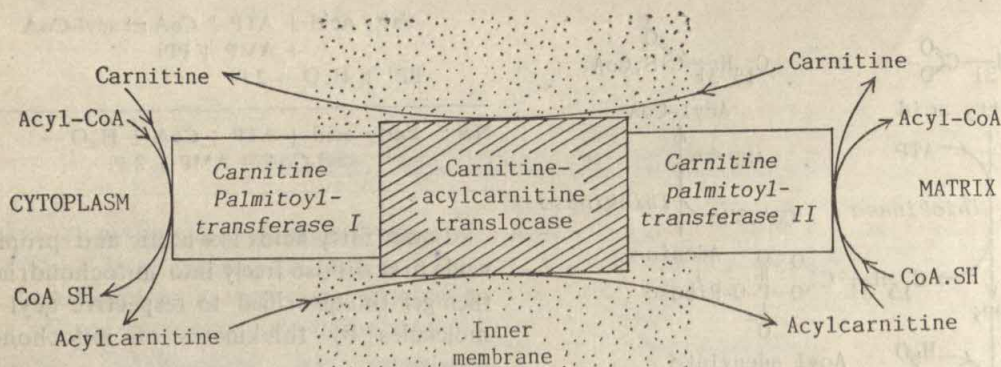


Fig. 13.4. Transport of acylcarnitine in exchange of carnitine across the inner mitochondrial membrane.

of *carnitine palmitoyltransferase II* located on the matrix surface of the inner membrane. This produces acyl-CoA and free carnitine in the matrix. Carnitine is translocated back to the cytoplasm in exchange of another acylcarnitine molecule brought into the matrix.

Carnitine deficiency: This results from either a low rate of carnitine biosynthesis or an enhanced urinary loss of carnitine or its loss in hemodialysis. The resulting decline in β -oxidation leads to fat accumulation in muscles, liver and other tissues, muscular weakness due to reduced energy output from fatty acids, poor formation of ketone bodies, reduced gluconeogenesis and hypoglycemia. New-born infants are more susceptible to carnitine deficiency. It is remedied by feeding carnitine.

Reaction sequence of β -oxidation

Beta-oxidation of a saturated acyl-CoA consists of a cyclic repetition of four reactions in the mitochondrial matrix. Each cycle shortens the fatty acid chain by releasing two carboxyl-terminal carbons as acetyl-CoA (Fig. 13.5).

(a) A flavoprotein called *acyl-CoA dehydrogenase* oxidizes acyl-CoA to Δ^2 -trans-enoyl-CoA (*trans*- α,β -unsaturated acyl-CoA)

by transferring hydrogens from C^2 and C^3 of the acyl group to the FAD prosthetic group. FAD is reduced thereby to $FADH_2$ which is reoxidized to FAD by a transfer of its electrons to an *electron-transporting flavoprotein* (ETF) through an iron-sulfur protein. ETF in turn transfers the electrons to coenzyme Q. Two high-energy ATP bonds are produced as the electrons flow from coenzyme Q to molecular O_2 through the mitochondrial respiratory chain.

Acyl-CoA dehydrogenases possess chain-length specificity, each acting on acyl groups of specific chain-lengths.

A genetic deficiency of the mitochondrial acyl-CoA dehydrogenase for medium-chain fatty acids decreases their β -oxidation; they are instead increasingly oxidized by ω -oxidation to form α,ω -dicarboxylic fatty acids (C_6-C_{10}) which are eliminated in large amounts in the urine (*dicarboxylic aciduria*).

(b) The C_2-C_3 double-bond of Δ^2 -trans-enoyl-CoA is saturated with water by Δ^2 -enoyl-CoA hydratase (crotonase). This changes Δ^2 -trans-enoyl-CoA to L(+)-3-hydroxyacyl-CoA. The enzyme can hydrate acyl groups, varying largely in chain-length. It has no *cis-trans* specificity for its substrates and can also hydrate Δ^2 -cis-enoyl-CoA into D(-)-3-hydroxyacyl-CoA.

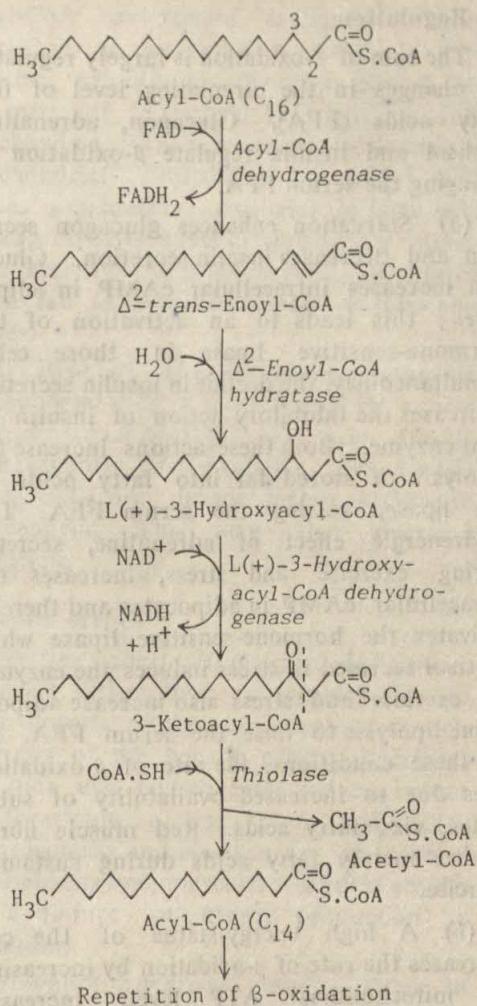


Fig. 13.5. Steps of beta-oxidation.

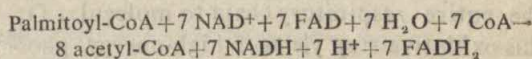
(c) L(+)-3-Hydroxyacyl-CoA dehydrogenase oxidizes L(+)-3-hydroxyacyl-CoA to 3-ketoacyl-CoA by transferring H⁺ and electrons from the C³-hydroxyl group of the substrate to NAD⁺. NADH, thus produced from NAD⁺, is reoxidized to the latter by a transfer of its reducing equivalents to the mitochondrial respiratory chain. The flow of the electrons to molecular O₂ through the chain generates 3 high-energy ATP bonds.

L(+)-3-Hydroxyacyl-CoA dehydrogenase possesses D-L specificity and acts on only the

L isomer of the hydroxyacyl group. It shows no chain-length specificity.

(d) β -Ketothiolase (thiolase) uses coenzyme A to cleave 3-ketoacyl-CoA by thiolysis into acetyl-CoA and a new acyl-CoA shorter than the original one by two carbons. The enzyme has no chain-length specificity.

This cycle of four reactions is then repeated with the new acyl-CoA. Recurrence of this cycle ultimately breaks an even-C fatty acid into many acetyl-CoA molecules, but an odd-C fatty acid into one propionyl-CoA and many acetyl-CoA molecules. Palmitic acid (C₁₆) is thus oxidized into 8 acetyl-CoA molecules through 7 cycles of β -oxidation. Following is the stoichiometry of palmitate oxidation.



*Acetyl-CoA molecules are normally oxidized to CO₂ and H₂O in the TCA cycle. But if the TCA cycle fails to oxidize all the acetyl-CoA molecules, they are condensed in pairs to produce acetoacetyl-CoA and ketone bodies.

Propionyl-CoA from odd-C acids is changed to succinyl-CoA for metabolism through the TCA cycle.

Peroxisomes contain a special *peroxisomal* β -oxidation system. A flavoprotein aerobic dehydrogenase of this system oxidizes specifically the CoA thioesters of very long (C₂₀ to C₄₀) fatty acids. Each cycle of this oxidation yields an acetyl-CoA, H₂O₂ and an acyl-CoA shorter by C₂ than the preceding one, until octanoyl-CoA (C₈) is produced. A *carnitine shuttle* then transfers octanoyl groups from peroxisomes to cytoplasm and they are subsequently oxidized by the mitochondrial β -oxidation system.

An even-C dicarboxylic acid produces through repeated β -oxidation many acetyl-CoA molecules and finally a dicarboxylic acyl-CoA

molecule such as suberyl-CoA (C_8), adipoyl-CoA (C_6) and succinyl-CoA.

Energetics

Each β -oxidation cycle has only two oxidative steps, catalyzed respectively by acyl-CoA dehydrogenase and L(+)-3-hydroxyacyl-CoA dehydrogenase. $FADH_2$ produced in the first case, donates its electrons to coenzyme Q of the mitochondrial respiratory chain. Only 2 high-energy phosphate bonds are produced by the flow of these electrons to molecular O_2 along the chain, because the electrons bypass the NADH-Q reductase complex of the chain (page 302). In the second case, NADH produced donates its electrons to the NADH-Q reductase complex of the chain. As these electrons flow through the entire respiratory chain to molecular O_2 , this oxidative step yields 3 high-energy phosphate bonds. So, each cycle of β -oxidation produces 5 high-energy bonds of ATP. In addition, each acetyl-CoA molecule is oxidized through the TCA cycle, producing 12 high-energy phosphate bonds (page 331).

During the oxidation of an even-C fatty acid bearing n number of carbons, β -oxidation cycles occur $\frac{1}{2}n - 1$ number of times and produce $\frac{1}{2}n$ number of acetyl-CoA molecules. But irrespective of chain length, 2 high-energy bonds of ATP are spent in the initial thioesterification of the fatty acid to acyl-CoA by thiokinase. Thus, the net number of high-energy phosphate bonds, gained by the oxidation of the fatty acid (C_n), is given by: $5(\frac{1}{2}n - 1) + 12 \times \frac{1}{2}n - 2$. For example, oxidation of one mole of palmitate (C_{16}) yields: $5(\frac{1}{2} \times 16 - 1) + 12(\frac{1}{2} \times 16) - 2 = 129$ moles of high-energy bonds. This is equivalent to 7.3×129 or 940 kcal because $\Delta G^{\circ'}$ of hydrolysis of ATP amounts to -7.3 kcal mol^{-1} . As the $\Delta G^{\circ'}$ for complete combustion of palmitate amounts to -2340 kcal mol^{-1} , the efficiency of energy gain by fatty acid oxidation amounts to $100 \times 940 / 2340 \approx 40\%$.

Regulation

The rate of β -oxidation is largely regulated by changes in the circulating level of free fatty acids (FFA). Glucagon, adrenaline, cortisol and insulin regulate β -oxidation by changing the serum FFA.

(a) Starvation enhances glucagon secretion and decreases insulin secretion. Glucagon increases intracellular cAMP in adipocytes; this leads to an activation of the hormone-sensitive lipase in those cells. Simultaneously, the decline in insulin secretion decreases the inhibitory action of insulin on that enzyme. Both these actions increase the lipolysis of stored fat into fatty acids by the lipase, raising the serum FFA. The β -adrenergic effect of adrenaline, secreted during exercise and stress, increases the intracellular cAMP in adipocytes and thereby activates the hormone-sensitive lipase while cortisol secreted in stress induces the enzyme. So, exercise and stress also increase adipose tissue lipolysis to raise the serum FFA. In all these conditions, the rate of β -oxidation rises due to increased availability of substrates, viz., fatty acids. Red muscle fibres oxidize mainly fatty acids during sustained exercise.

(b) A high energy-status of the cell decreases the rate of β -oxidation by increasing the mitochondrial ATP level. Increased mitochondrial ATP reduces the rate of the TCA cycle, leading to the accumulation of acetyl CoA in mitochondria. As the inner mitochondrial membrane is impermeable to coenzyme A, mitochondrial accumulation of acetyl-CoA decreases the availability of free coenzyme A for running the β -oxidation, thus reducing the rate of the latter.

(c) A high ATP level is associated with a rise in cellular citrate level. Citrate provides more acetyl-CoA in the cytoplasm with the help of ATP-citrate lyase and also allosterically activates acetyl-CoA carboxylase. So,

acetyl-CoA carboxylase carboxylates more acetyl-CoA to malonyl-CoA which inhibits carnitine palmitoyltransferase I needed for the entry of long-chain acyl groups into mitochondria. The consequent decline in the mitochondrial availability of fatty acids reduces β -oxidation. Any rise in fatty acid synthesis involves a rise in acetyl-CoA carboxylase activity and is consequently associated with a fall in β -oxidation rate in liver and muscles.

(d) Rise in cellular energy-status or mitochondrial ATP is accompanied by a rise in mitochondrial NADH/NAD⁺ ratio. NAD⁺ is consequently less available for L(+)-3-hydroxyacyl-CoA dehydrogenase action in mitochondria. This may also decrease β -oxidation.

Inborn errors

Carnitine palmitoyltransferase deficiencies may affect either liver or muscles due to genetic defects of the respective isoenzymes. Deficiency of the hepatic enzyme decreases β -oxidation and consequently reduces ketone body formation, plasma ketone body level, gluconeogenesis and blood sugar. In case of the muscle enzyme, muscular weakness results from a failure of energy production by β -oxidation.

Zellweger's syndrome results from an inborn absence of peroxisomes. Very long polyunsaturated fatty acids fail to be oxidized and accumulate in the brain.

13.2 KETONE BODIES

Acetoacetic acid, 3-hydroxybutyric acid (β -hydroxybutyrate) and acetone are collectively known as ketone bodies.

Formation

Ketone bodies are formed in the liver. Increased β -oxidation produces ketone bodies. Knoop originally proposed that the last C₄ fragment, left after repeated β -oxidation of

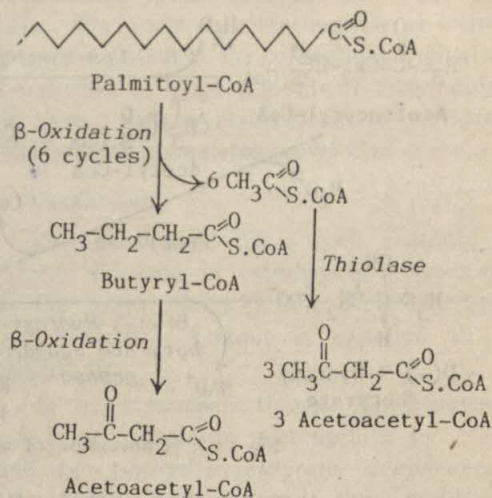


Fig. 13.6. Formation of acetoacetyl-CoA from palmitoyl-CoA.

even-C fatty acids, is changed to acetoacetate through the steps of β -oxidation. But this falls far short of the actual amount of ketone bodies formed during the β -oxidation of a fatty acid. It has also been shown that all the carbons of an even-C fatty acid find their way into acetoacetate molecules. Thus, acetoacetyl-CoA is produced not only by the β -oxidation of the last C₄ fragment (viz., butyryl-CoA) of the fatty acid, but also by the *thiolase*-catalyzed condensation of pairs of acetyl groups from acetyl CoA molecules released by the cycles of β oxidation (Fig. 13.6).

HMG-CoA synthase of hepatic mitochondria condenses most of the acetoacetyl-CoA with acetyl-CoA to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) which is next cleaved by *HMG-CoA lyase* into acetoacetate and acetyl-CoA (Fig. 13.7). The acetyl-CoA molecule thus released does not carry the carbons of the original acetyl-CoA molecule used by HMG-CoA synthase; instead, it inherits its methyl and carboxyl carbons from respectively the alpha and carboxyl carbons of the acetoacetyl CoA molecule originally used. Thus, the acetoacetate finally formed

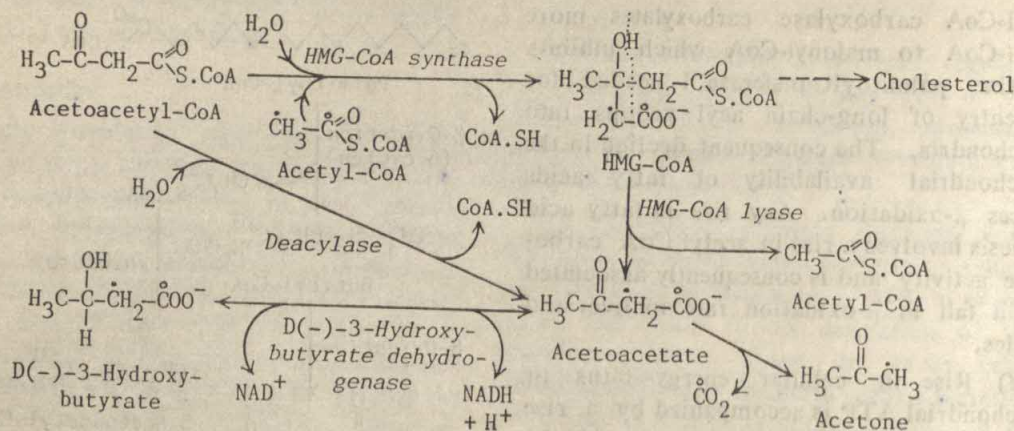


Fig. 13.7. Conversion of acetoacetyl-CoA to ketone bodies.

gets its carboxyl (C^1) and methylene (C^2) carbons from the acetyl-CoA initially used, and its ketonyl (C^3) and methyl (C^4) carbons from the acetoacetyl-CoA. HMG-CoA synthase is probably the rate-limiting enzyme for ketone body formation.

Besides, a *deacylase* may directly hydrolyze small amounts of acetoacetyl-CoA into coenzyme A and acetoacetate.

In hepatic mitochondria, D(-)-3-hydroxybutyrate dehydrogenase uses NADH to reduce most acetoacetate into D(-)-3-hydroxybutyrate. Small amounts of acetoacetate may be spontaneously decarboxylated to acetone. 3-Hydroxybutyrate and acetoacetate are carried by blood to extrahepatic tissues for disposal because the liver cannot reconvert acetoacetate to acetoacetyl-CoA for further catabolism.

Significance :

(a) In starvation or diabetes, or on a high-fat low-carbohydrate diet, mainly fatty acids are oxidized instead of carbohydrates for energy purposes. But the rate of the TCA cycle falls in these states due to a decline in mitochondrial oxaloacetate in consequence of increased gluconeogenesis and reduced carbohydrate catabolism. So, acetyl-CoA from enhanced β -oxidation accumulates

in hepatic mitochondria. Because the inner mitochondrial membrane is impermeable to coenzyme A, rise in mitochondrial acetyl-CoA would tend to reduce the amount of free coenzyme A available for β -oxidation. By forming acetoacetate from acetyl-CoA, the liver endeavours to restore the amount of free coenzyme A in mitochondria for the β -oxidation to continue.

(b) During starvation, liver depends largely for its energy source on the β -oxidation of fatty acids derived from adipose tissue lipolysis, and consequently produces plenty of ketone bodies. These are supplied to the brain, heart, muscles and kidneys, and become their major fuel for energy production, particularly in prolonged starvation. This metabolic shift towards the formation and use of ketone bodies, has been called *ketosis*.

Regulation :

Ketogenesis is regulated mainly by controlling the activity of HMG-CoA synthase, the rate-limiting enzyme for the process. In starvation, HMG-CoA synthase activity is directly enhanced by the heightened mitochondrial concentration of its substrate acetyl-CoA from increased β -oxidation. This enhances ketogenesis. On the contrary, carbohydrate

oxidation increases the mitochondrial concentration of succinyl-CoA which succinylates the acetate-binding site of HMG-CoA synthase to inhibit the latter; this decreases ketogenesis.

Glucagon, secreted in starvation, reduces the hepatic mitochondrial concentrations of oxaloacetate and succinyl-CoA by directly stimulating hepatic gluconeogenesis. Glucagon also increases adipose tissue lipolysis to enhance the amount of fatty acids reaching the liver from adipose tissue; this leads to increased β -oxidation and higher mitochondrial concentration of acetyl-CoA. Enhanced β -oxidation also increases the mitochondrial NADH/NAD⁺ ratio, and consequently increases the NADH-mediated reduction of oxaloacetate to malate; the resulting non-availability of oxaloacetate for condensation with acetyl-CoA prevents the entry of the latter in the TCA cycle and enhances its accumulation in mitochondria. The simultaneous decline in mitochondrial succinyl-CoA and rise in mitochondrial acetyl-CoA enable the acetyl group to replace the succinyl group bound with HMG-CoA synthase and consequently reverses the inhibition of the latter. This increases ketogenesis.

Diabetic ketosis results largely from a lack of insulin action. Increased gluconeogenesis reduces the mitochondrial concentration of

oxaloacetate. The rate of the TCA cycle falls. There is a simultaneous rise in adipose tissue lipolysis with the consequent arrival and β -oxidation of large amounts of fatty acids in the liver. Ketosis results from the failure to oxidize the accumulated acetyl-CoA.

Catabolism

Ketone bodies, other than acetone, are largely oxidized in extrahepatic tissues such as the brain, heart muscle, striated muscles and kidneys. Acetone is expelled in the expired air.

In the extrahepatic tissues, 3-hydroxybutyrate is reoxidized to acetoacetate by NAD⁺ and D(-)-3-hydroxybutyrate dehydrogenase (Fig. 13.7). Acetoacetate is then reactivated by thioesterification to acetoacetyl-CoA in either of two ways. (i) *Acetoacetyl-CoA synthase* changes acetoacetate to acetoacetyl-CoA with the help of coenzyme A and ATP, the latter being hydrolyzed simultaneously to AMP and PPi (Fig. 13.8). The reaction is made to proceed towards acetoacetyl-CoA by the immediate hydrolysis of PPi to Pi molecules by an *inorganic pyrophosphatase*. (ii) *Succinyl-CoA-acetoacetate CoA transferase* (thiophorase) may catalyze the transfer of the coenzyme A molecule from succinyl-CoA to acetoacetate.

Acetoacetyl-CoA is next thiolized into two acetyl-CoA molecules by *thiolase* and

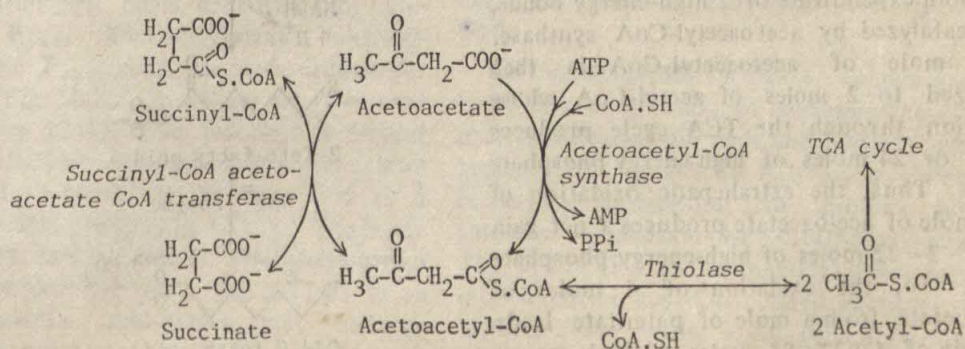


Fig. 13.8. Catabolism of acetoacetate.

coenzyme A. Acetyl-CoA is then oxidized through the TCA cycle.

During severe ketosis, extrahepatic tissues cannot remove all the ketone bodies from the blood. This increases the urinary elimination of ketone bodies (*ketonuria*). As much as 20 and 100 g of ketone bodies may be excreted in 24 hours' urine during starvation and diabetes respectively. As 3-hydroxybutyrate cannot directly condense with coenzyme A, its metabolism is slower than that of acetoacetate. Moreover, most of the acetoacetate is reduced to 3-hydroxybutyrate before their release from the liver. So, 3-hydroxybutyrate is the major ketone body in blood and urine.

Energetics

Seven cycles of β -oxidation break each mole of palmitate (C_{16}) to 8 moles of acetyl-CoA, which are changed by ketogenesis into 4 moles of acetoacetate (C_4). Because 5 high-energy phosphate bonds are produced in each cycle of β -oxidation and 2 high-energy bonds are spent initially in thioesterifying palmitate to palmitoyl-CoA, the conversion of each mole of palmitate to 4 moles of acetoacetate causes a gain of $7 \times 5 - 2 = 33$ moles of high-energy phosphate bonds; in contrast, 129 moles of such bonds are gained per mole of palmitate completely oxidized to CO_2 and H_2O .

In extrahepatic tissues, thioesterification of each mole of acetoacetate to acetyl-CoA incurs an expenditure of 2 high-energy bonds, when catalyzed by acetoacetyl-CoA synthase. Each mole of acetoacetyl-CoA is then thiolized to 2 moles of acetyl-CoA whose oxidation through the TCA cycle produces 2×12 or 24 moles of high-energy phosphate bonds. Thus, the extrahepatic oxidation of each mole of acetoacetate produces a net gain of $24 - 2 = 22$ moles of high-energy phosphate bonds. So, the oxidation of 4 moles of acetoacetate from a mole of palmitate leads to a gain of $4 \times 22 = 88$ moles of high-energy phosphate bonds.

13.3 ALPHA AND OMEGA OXIDATIONS

(a) Brain tissue contains ferroproteins called α -hydroxylases (2-hydroxylases) which aerobically oxidize some saturated or unsaturated, even-C, long-chain fatty acids with the help of an electron-donor cofactor like L-ascorbate or tetrahydrobiopterin. α -Hydroxylases act directly on the fatty acids and not on their coenzyme A thioesters. Their action hydroxylates the C^2 (α -C) of the fatty acid, forming the corresponding L-(+)-2-hydroxyfatty acid (Fig. 13.9). α -Oxidations do not produce high-energy bonds. (i) In the brain,

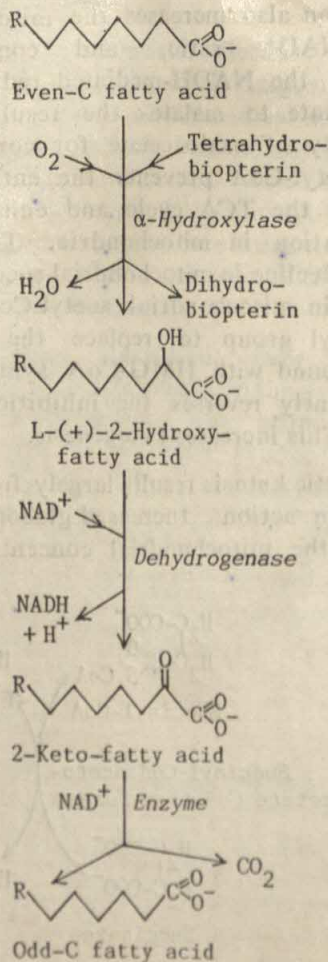


Fig. 13.9. Alpha-oxidation of fatty acids.

α -oxidation produces long-chain 2-hydroxy fatty acids for incorporation into cerebrosides and sulfatides. For example, lignocerate, a saturated C_{24} acid, undergoes α -oxidation to form cerebronic acid (2-hydroxylignocerate) which is incorporated into cerebrosides called cerebros; nervonate, a monounsaturated C_{24} fatty acid, is similarly oxidized to 2-hydroxynervonate to be incorporated into cerebrosides called oxynervons, (ii) Some 2-hydroxy acids, produced by α -hydroxylase action, may be further oxidized by an NAD^+ -dependent dehydrogenase to produce the corresponding 2-keto (α -keto) acids which are then decarboxylated to yield odd-C fatty acids for incorporation into brain sphingolipids (Fig. 13.9). (iii) α -Hydroxylase action helps to remove the C^3 -methyl group of phytanic acid produced from dietary phytol. The product is then catabolized by β -oxidation.

In *Refsum's disease* caused by a genetic deficiency of α -hydroxylase, failure of phytanate oxidation raises serum and tissue phytanates and produces neurological symptoms.

(b) ω -Hydroxylases occur in hepatic microsomes. Small amounts of medium- and long-chain fatty acids undergo ω -oxidation to the corresponding ω -hydroxy acids by ω -hydroxylases in the smooth endoplasmic reticulum of the liver (Fig. 13.10). ω -Hydroxylase consists of a microsomal cytochrome P_{450} oxygenase system; the latter is an electron-transport chain constituted by cytochrome P_{450} , FAD-dependent NADPH-cytochrome P_{450} reductase and iron-sulfur protein (Fig. 10.3). It utilizes the electrons donated by NADPH in reducing molecular O_2 to water and simultaneously hydroxylates the ω -methyl group of the fatty acid to a hydroxymethyl group (Fig. 13.10). The latter is oxidized further into an ω -carboxyl group by other oxidoreductases. The product is an α,ω -dicarboxylic acid which may undergo repeated β -oxidation to produce several molecules of acetyl-CoA and the CoA thioesters of

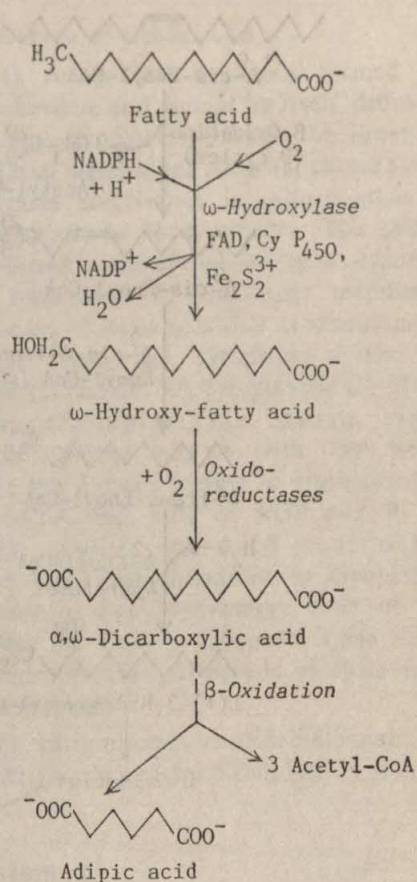


Fig. 13.10. Omega-oxidation of fatty acids.

either suberic (C_8) or adipic (C_6) acids. ω -Hydroxylase action does not require the initial thioesterification of fatty acids to acyl-CoA.

In *dicarboxylic aciduria* characterized by urinary elimination of medium-chain (C_6 to C_{10}) dicarboxylic acids and hypoglycemia, medium- and long-chain fatty acids undergo increased ω -oxidation to produce large amounts of medium-chain dicarboxylic acids. The defect lies in an inherited deficiency of mitochondrial acyl-CoA dehydrogenases for medium-chain acids, a consequent failure in their β -oxidation and a corresponding rise in ω -oxidation.

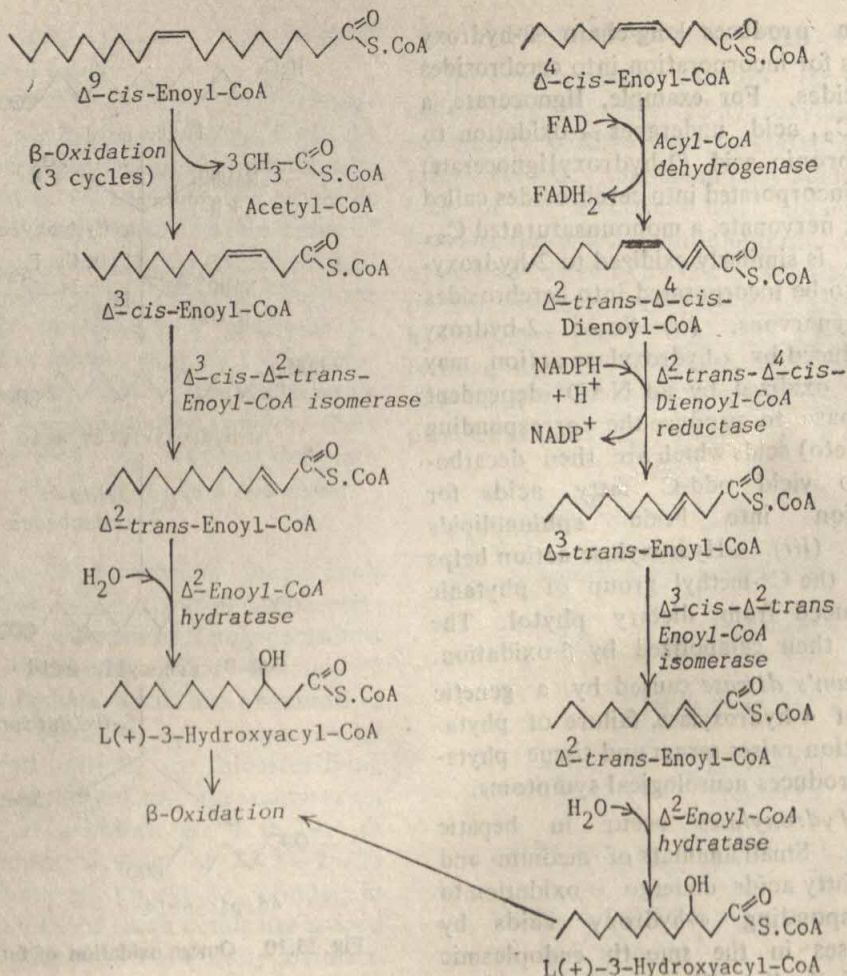


Fig. 13.11. Oxidation of unsaturated fatty acids,

13.4 OXIDATION OF UNSATURATED FATTY ACIDS

Unsaturated fatty acids are also oxidized mainly by β -oxidation. But each double-bond has to be changed by one of the following methods before β -oxidation can continue.

(a) If a Δ^3 -cis-enoyl-CoA is formed during β -oxidation, it is first isomerized to the corresponding Δ^2 -trans-enoyl-CoA by Δ^3 -cis- Δ^2 -trans-enoyl-CoA isomerase (Fig. 13.11). The enzyme not only shifts the Δ^3 double-bond to the Δ^2 position but also acts simultaneously as a *cis-trans* isomerase. Δ^2 -Enoyl-

CoA hydratase of the β -oxidation pathway then hydrates Δ^2 -trans-enoyl-CoA to L(+)-3-hydroxyacyl-CoA, thus making the reactions of β -oxidation continue again.

(b) If a Δ^4 -cis-enoyl-CoA is produced at any stage of β -oxidation, it is first oxidized to Δ^2 -trans- Δ^4 -cis-dienoyl-CoA by acyl-CoA dehydrogenase (Fig. 13.11); as in the case of a saturated acyl-CoA, the enzyme transfers hydrogens from C^3 and C^8 of Δ^4 -cis-enoyl CoA to the FAD prosthetic group, reducing the latter to FADH_2 . Next, Δ^2 -trans- Δ^4 -cis-dienoyl-CoA reductase reduces Δ^2 -trans- Δ^4 -

cis-dienoyl-CoA to Δ^3 -*trans*-enoyl-CoA, using NADPH as the electron-donor. Δ^3 -*trans*-Enoyl-CoA is then isomerized to Δ^2 -*trans*-enoyl-CoA by the Δ^3 -*cis*- Δ^2 -*trans*-enoyl-CoA isomerase mentioned above; in this case, the enzyme catalyzes only a positional shift of the double-bond but no *cis-trans* isomerization. Δ^2 -*trans*-Enoyl-CoA is then hydrated by Δ^2 -enoyl-CoA hydratase to L(+)-3-hydroxyacyl-CoA, thus continuing the β -oxidation reactions again.

13.5 CYTOPLASMIC SYNTHESIS OF FATTY ACIDS

A multienzyme system called *fatty acid synthase* catalyzes the total (de novo) synthesis of palmitate from acetyl-CoA in the cytosol of liver, lactating mammary glands, adipocytes, renal cortex, brain and lungs of mammals and birds. The process is a reductive synthesis and requires acetyl-CoA and HCO_3^- as the substrates, NADPH as the electron-donor cofactor and ATP as the source of energy.

Sources of acetyl-CoA

(a) Acetyl-CoA is largely formed inside mitochondria and cannot by itself diffuse out into the cytoplasm through the inner mitochondrial membrane. Instead, *citrate synthase* condenses acetyl-CoA in mitochondria with oxaloacetate to form citrate. The latter is transferred to the cytoplasm by a *tricarboxylate transporter* of the inner membrane in exchange of malate which is simultaneously carried from the cytoplasm to the mitochondrial matrix. A cytoplasmic C—C lyase called *ATP-citrate lyase* (citrate cleavage enzyme) cleaves citrate with the help of ATP; this breaks citrate to oxaloacetate and acetyl-CoA, and ATP to ADP and Pi (Fig. 13.12). In effect, one acetyl group is transferred from mitochondrion to cytoplasm at the cost of one high-energy bond of ATP. Because of poor ATP-citrate lyase activity in ruminants, this pathway is of little importance in them.

(b) In ruminants, considerable amounts of acetate are derived from cellulose fermentation

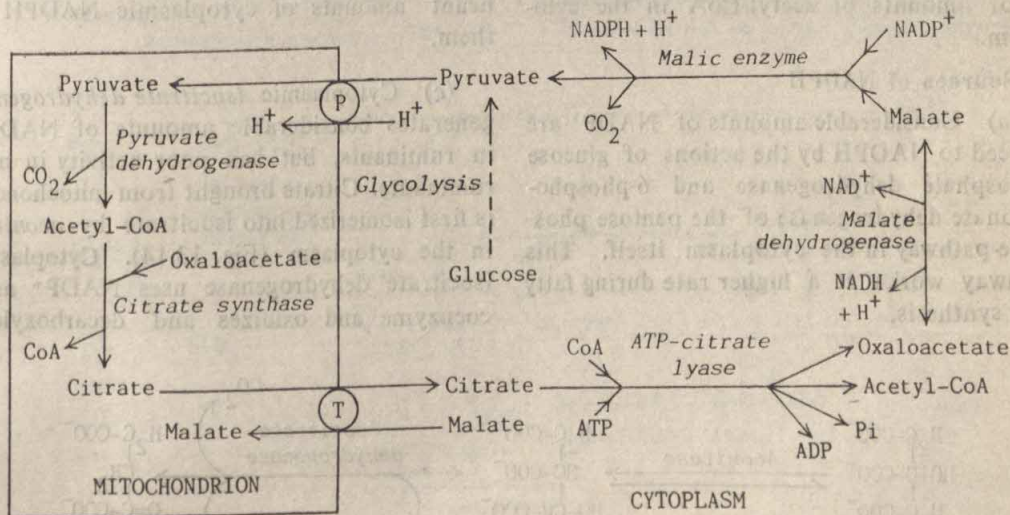


Fig. 13.12. ATP-citrate lyase and malic enzyme in providing acetyl-CoA and NADPH for fatty acid synthesis. P: pyruvate transporter; T: tricarboxylate transporter.

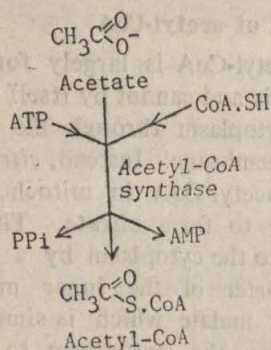


Fig. 13.13. Action of acetyl-CoA synthase.

and enter the cytoplasm from extracellular fluids. *Acetyl-CoA synthase* (acetate thio-kinase) then uses coenzyme A and ATP to thioesterify acetate to acetyl-CoA in the cytoplasm (Fig. 13.13).

(c) *Carnitine acetyltransferase* may probably transfer acetyl group from acetyl-CoA to carnitine to produce acetylcarnitine in mitochondria. After translocation to cytoplasm across the inner membrane, acetyl-carnitine may transfer its acetyl group to coenzyme A to form acetyl-CoA in the cytoplasm. But this may at most provide only minor amounts of acetyl-CoA in the cytoplasm.

Sources of NADPH

(a) Considerable amounts of NADP^+ are reduced to NADPH by the actions of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of the pentose phosphate pathway in the cytoplasm itself. This pathway works at a higher rate during fatty acid synthesis.

(b) Oxaloacetate, produced from citrate by ATP-citrate lyase, is reduced to malate by NADH and cytoplasmic *malate dehydrogenase* (Fig. 13.12). Another cytoplasmic enzyme called *malic enzyme* (NADP^+ -malate dehydrogenase) oxidizes as well as decarboxylates malate to pyruvate; NADP^+ is used in this reaction as the acceptor of reducing equivalents (H^+ and e) from malate and is thereby reduced to NADPH. Pyruvate is carried into mitochondria by an inner membrane carrier, depending on the inward H^+ concentration gradient. In effect, the combined action of malate dehydrogenase and malic enzyme transfers reducing equivalents from NADH to NADPH in the cytoplasm. This is another major source of NADPH in nonruminants. Synthesis of one palmitate molecule requires 14 NADPH molecules as donors of reducing equivalents; of these, 8 NADPH molecules are usually generated by this pathway while the remaining 6 molecules are provided by the pentose phosphate pathway. But ATP-citrate lyase and malic enzyme have very poor activities in ruminants; so, this pathway does not produce significant amounts of cytoplasmic NADPH in them.

(c) Cytoplasmic *isocitrate dehydrogenase* generates considerable amounts of NADPH in ruminants, but has poor activity in non-ruminants. Citrate brought from mitochondria is first isomerized into isocitrate by *aconitase* in the cytoplasm (Fig. 13.14). Cytoplasmic isocitrate dehydrogenase uses NADP^+ as its coenzyme and oxidizes and decarboxylates

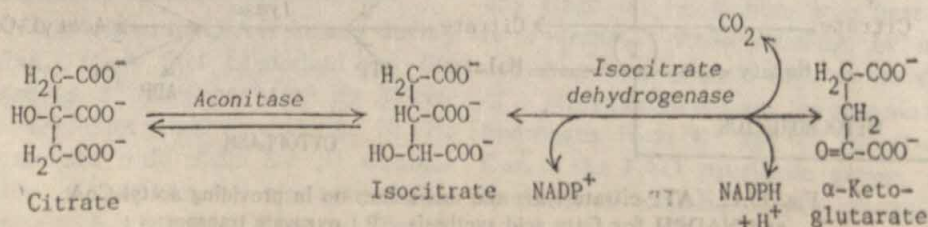


Fig. 13.14. Cytoplasmic isocitrate dehydrogenase in forming NADPH for fatty acid synthesis.

isocitrate to α -ketoglutarate; NADP^+ gets simultaneously reduced to NADPH .

Acetyl-CoA carboxylase

Except the first molecule of acetyl-CoA used as the primer in the cytoplasmic synthesis of palmitate, all other acetyl-CoA molecules used in the process are first carboxylated to malonyl-CoA by an ATP-dependent, cytoplasmic C—C ligase called *acetyl-CoA carboxylase*. It is a multienzyme system consisting of a biotin carboxyl carrier protein, biotin carboxylase and a transcarboxylase. It carries biotin as the prosthetic group. As in cases of pyruvate carboxylase and propionyl-CoA carboxylase, the $\epsilon\text{-NH}_2$ of a lysine residue of the *biotin carboxyl carrier protein* remains covalently bound by an amide bond to the sidechain carboxyl group of biotin (page 154). In the first step of a ping-pong type of reaction, the *biotin carboxylase* component of the enzyme catalyzes the carboxylation of the enzyme-bound biotin to form an enzyme-bound carboxybiotin intermediate with the help of HCO_3^- (as a source of CO_2) and ATP (Fig. 13.15).

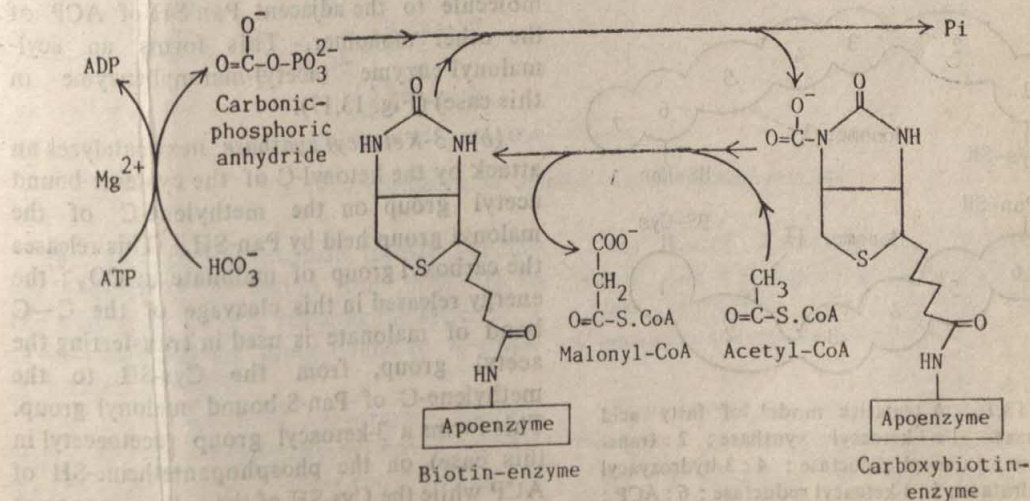
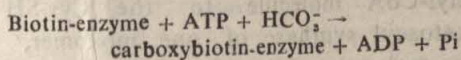
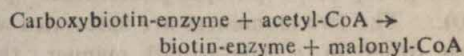


Fig. 13.15. Action of acetyl-CoA carboxylase.

This involves the loss of a high-energy bond of ATP and an initial formation of a carbonic-phosphoric anhydride from which the carboxylate ion is transferred to biotin. In the second step of the ping-pong reaction, the *transcarboxylase* component of the enzyme transfers the activated carboxyl group from carboxybiotin to acetyl-CoA, changing the latter to malonyl-CoA.



Acetyl-CoA carboxylase is the *rate-limiting enzyme* for fatty acid biosynthesis. It belongs to the M series of allosteric enzymes. Citrate, its allosteric activator, changes its inactive monomeric form to active polymeric forms and manifold increases its V_{max} . Palmitoyl-CoA, resulting from fatty acid synthesis, inhibits the enzyme by converting it to the inactive monomer.

Fatty acid synthase

This is another multienzyme complex in the cytoplasm of yeast, mammalian and avian cells. It is an ellipsoid dimer of two identical polypeptide monomers and measures about $25 \text{ nm} \times 21 \text{ nm}$ in yeast. Six enzymes and one

acyl-carrier protein (ACP) are covalently linked in the following order from end to end in each monomer chain: 3-ketoacyl synthase, transacylase, enoyl reductase, 3-hydroxyacyl dehydratase, 3-ketoacyl reductase, ACP and thioesterase. At one end of each monomer, 3-ketoacyl synthase carries a cysteine residue; the ACP near its other end bears a prosthetic group of 4'-phosphopantetheine (page 160). The two monomers are associated with each other in a head-to-tail manner; thus, the cysteine-SH of one monomer lies close to the phosphopantetheine-SH (Pan-SH) of the other and vice versa (Fig. 13.16). The individual monomers are inactive when isolated from each other. But in the dimer form, the monomers jointly synthesize two palmitate molecules simultaneously at the two ends of the dimer; at each end, one palmitate molecule is synthesized by the coordinated actions of the 3-ketoacyl synthase and transacylase of one monomer and of the thioesterase, ACP, 3-ketoacyl reductase, 3-hydroxyacyl dehydratase and enoyl reductase of the other monomer; this involves the participation of the Cys-SH of one monomer and the Pan-SH of the other. The

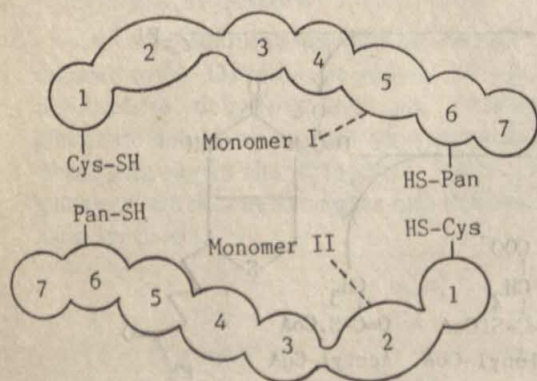


Fig. 13.16. A tentative model of fatty acid synthase. 1: 3-ketoacyl synthase; 2: transacylase; 3: enoyl reductase; 4: 3-hydroxyacyl dehydratase; 5: 3-ketoacyl reductase; 6: ACP; 7: thioesterase.

dimer thus seems to act as two units, each constituted by a part of either monomer.

The occurrence of all enzymes of the synthase in a macromolecular complex ensures their uninterrupted sequential actions right upto the completion of the synthesis. The Pan-SH holds the growing acyl group during the process and the 2 nm long, flexible arm of ACP-phosphopantetheine brings the acyl group successively to the different enzyme sites preventing its diffusion away from the synthase or its diversion to other competing reactions.

In contrast to the multienzyme fatty acid synthase complex of mammals, individual enzymes of the fatty acid synthase system remain separate from each other as also from ACP in bacteria (*E. coli*), *Euglena gracilis* and potato tubers. ACP carries the growing acyl group as acyl-ACP to these enzymes in succession.

Reaction sequence :

(a) *Transacylase* of the multienzyme complex transfers the acetyl group from an acetyl-CoA molecule to the Cys-SH of 3-ketoacyl synthase of one monomer, and the malonyl group from a malonyl-CoA molecule to the adjacent Pan-SH of ACP of the other monomer. This forms an acyl-malonyl-enzyme (acetyl-malonyl-enzyme in this case) (Fig. 13.17).

(b) *3-Ketoacyl synthase* next catalyzes an attack by the ketonyl-C of the cysteine-bound acetyl group on the methylene-C of the malonyl group held by Pan-SH. This releases the carboxyl group of malonate as CO_2 ; the energy released in this cleavage of the C—C bond of malonate is used in transferring the acetyl group, from the Cys-SH to the methylene-C of Pan-S-bound malonyl group. This forms a 3-ketoacyl group (acetoacetyl in this case) on the phosphopantetheine-SH of ACP while the Cys-SH of the other monomer becomes free.

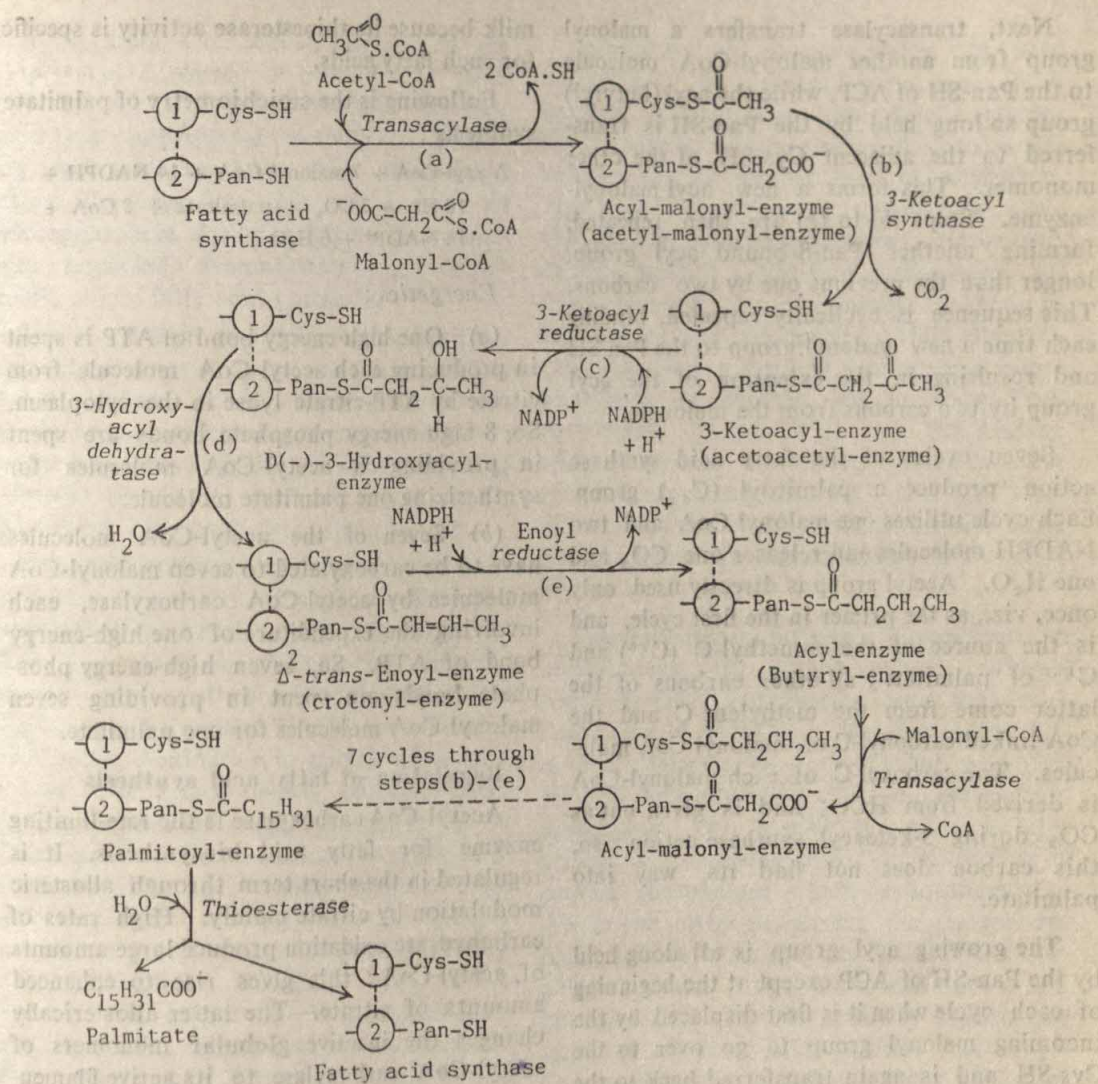


Fig. 13.17. Synthesis of palmitate by fatty acid synthase.

(c) *3-Ketoacyl reductase* transfers reducing equivalents (H^+ and e) from NADPH to the 3-ketoacyl group, reducing the latter to a D(-)-3-hydroxyacyl group bound to 4'-phosphopantetheine-SH.

(d) *3-Hydroxyacyl dehydratase* removes H_2O from D(-)-3-hydroxyacyl group, changing the latter to a Δ^2 -trans-enoyl (crotonyl) group.

(e) *Enoyl reductase* uses NADPH to reduce the Δ^2 -trans-enoyl group to an acyl group (butyryl in this case) still held by 4'-phosphopantetheine of ACP.

This completes the first cycle of the reactions. In effect, it adds two carbons from a malonyl group to an acetyl group (C_2), changing the latter to a C_4 acyl group.

Next, transacylase transfers a malonyl group from another malonyl-CoA molecule to the Pan-SH of ACP, while the acyl (butyryl) group so long held by the Pan-SH is transferred to the adjacent Cys-SH of the other monomer. This forms a new acyl-malonyl-enzyme. Steps (b) to (e) are then repeated, forming another Pan-S-bound acyl group, longer than the previous one by two carbons. This sequence is cyclically repeated, binding each time a new malonyl group to the Pan-SH and resulting in the extension of the acyl group by two carbons from the malonate.

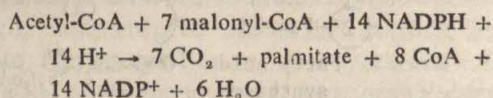
Seven cycles of the fatty acid synthase action produce a palmitoyl (C_{16}) group. Each cycle utilizes one malonyl-CoA and two NADPH molecules and releases one CO_2 and one H_2O . Acetyl group is directly used only once, viz., as the primer in the first cycle, and is the source of the ω -methyl-C (C^{16}) and C^{15} of palmitate; all other carbons of the latter come from the methylene-C and the CoA-linked carbonyl-C of malonyl-CoA molecules. The carboxyl-C of each malonyl-CoA is derived from HCO_3^- and is given out as CO_2 during 3-ketoacyl synthase action; so, this carbon does not find its way into palmitate.

The growing acyl group is all along held by the Pan-SH of ACP except at the beginning of each cycle when it is first displaced by the incoming malonyl group to go over to the Cys-SH and is again transferred back to the new malonyl group held by the Pan-SH.

The palmitoyl group, formed at the end of seven cycles, is released as free palmitate from the Pan-SH of ACP by the hydrolytic action of *thioesterase* of the synthase complex. Thioesterase of the hepatic fatty acid synthase possesses substrate specificity for palmitoyl group. So, fatty acid synthase action ends at palmitate stage in the liver. But the mammary gland fatty acid synthase can form short-chain and medium-chain fatty acids of

milk because its thioesterase activity is specific for such fatty acids.

Following is the stoichiometry of palmitate synthesis :



Energetics :

(a) One high-energy bond of ATP is spent in producing each acetyl-CoA molecule from citrate by ATP-citrate lyase in the cytoplasm. So, 8 high-energy phosphate bonds are spent in providing 8 acetyl-CoA molecules for synthesizing one palmitate molecule.

(b) Seven of the acetyl-CoA molecules have to be carboxylated to seven malonyl-CoA molecules by acetyl-CoA carboxylase, each involving the expenditure of one high-energy bond of ATP. So, seven high-energy phosphate bonds are spent in providing seven malonyl-CoA molecules for one palmitate.

Regulation of fatty acid synthesis

Acetyl-CoA carboxylase is the rate-limiting enzyme for fatty acid biosynthesis. It is regulated in the short term through allosteric modulation by citrate mainly. High rates of carbohydrate oxidation produce large amounts of acetyl-CoA; this gives rise to enhanced amounts of citrate. The latter allosterically changes the inactive globular monomers of acetyl-CoA carboxylase to its active filamentous polymers with a heightened V_{max} . This increases the carboxylation of acetyl-CoA and consequently enhances the fatty acid synthesis. On the contrary, long-chain acyl-CoA molecules such as palmitoyl-CoA are antagonistic to citrate and depolymerize the active polymeric form of acetyl-CoA carboxylase to its inactive monomer. Palmitoyl-CoA also reduces the availability of acetyl-CoA in the cytoplasm by inhibiting the tricarboxylate transporter of the inner mitochondrial membrane and consequently reducing the transfer

of citrate from the mitochondrion to the cytoplasm as a source of acetyl-CoA. Both these actions of palmitoyl-CoA decrease acetyl-CoA carboxylase activity to reduce fatty acid synthesis in a short term way. Factors like high-fat diet and a lack of glycerophosphate for triacylglycerol synthesis cause acyl-CoA accumulation and consequently reduce fatty acid synthesis.

The short term regulation of acetyl-CoA carboxylase consists also of its reversible phosphorylation and dephosphorylation. Catecholamines and glucagon may inhibit the enzyme by promoting the phosphorylation of a specific site in its molecule while insulin may activate it through dephosphorylation.

Fatty acid synthesis may also be regulated by altering the activity of pyruvate dehydrogenase (PDH) which oxidatively decarboxylates pyruvate to acetyl-CoA, the substrate for fatty acid synthesis. In starvation and diabetes, acetyl-CoA and NADH are produced by enhanced β -oxidation in mitochondria and activate PDH kinase which phosphorylates and inactivates PDH. High-fat diet increases cellular acyl-CoA which inhibits the ATP/ADP transporter of the inner mitochondrial membrane, thus reducing the ATP outflow from mitochondria and consequently raising the mitochondrial ATP/ADP ratio; this activates PDH kinase to phosphorylate and inactivate PDH. So, starvation, diabetes and high-fat diet reduce fatty acid synthesis by decreasing the formation of acetyl-CoA from pyruvate. High-carbohydrate diet increases cellular pyruvate through enhanced glycolysis; pyruvate inhibits PDH kinase and consequently increases PDH activity, leading to a rise in acetyl-CoA formation as well as fatty acid synthesis.

Long term regulation of fatty acid synthesis is largely mediated by the induction of synthesis of acetyl-CoA carboxylase, fatty acid synthase, ATP-citrate lyase and the enzymes generating NADPH.

Adrenaline and glucagon reduce fatty acid biosynthesis in several ways. (a) They increase cAMP in adipocytes, thereby enhance lipolysis in the adipose tissue and consequently increase the circulating level of acyl-CoA. This leads to a rise in the cytoplasmic acyl-CoA level in the liver and adipose tissue and thereby inhibits acetyl-CoA carboxylase. (b) They increase the intracellular cAMP in hepatocytes and adipocytes; cAMP allosterically activates a protein kinase which phosphorylates a specific site in acetyl-CoA carboxylase to inhibit it. (c) On binding with membrane α -adrenergic receptors, the catecholamines may also activate a Ca^{2+} -calmodulin-dependent protein kinase which may phosphorylate and inhibit acetyl-CoA carboxylase.

Insulin increases fatty acid biosynthesis in several ways. (a) Insulin reduces adipose tissue lipolysis and thereby lowers the circulating level of acyl-CoA. The consequent fall in the cytoplasmic level of acyl-CoA molecules removes their inhibitory effect on acetyl-CoA carboxylase. (b) Insulin may also directly activate that enzyme either by activating a protein phosphatase which dephosphorylates the enzyme, or by promoting the phosphorylation of the latter at a site distinct from that phosphorylated by catecholamines and glucagon. (c) Insulin probably induces the synthesis of fatty acid synthase and acetyl-CoA carboxylase to enhance fatty acid biosynthesis. The amounts of both the enzymes rise in well-fed states with elevated insulin secretion, and fall in diabetes and starvation with a decline in the circulating insulin level. (d) Insulin induces the synthesis of ATP-citrate lyase to increase the cytoplasmic cleavage of citrate to acetyl-CoA, the substrate for acetyl-CoA carboxylase. (e) It may also enhance the formation of acetyl-CoA from pyruvate by promoting dephosphorylation and the consequent activation of pyruvate dehydrogenase. (f) It also induces the synthesis

of malic enzyme, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, and consequently increases the availability of NADPH for fatty acid biosynthesis. (g) Insulin increases the transport of glucose into adipocytes and thereby promotes glycolysis which leads to the provision of acetyl-CoA for fatty acid synthesis. A fall in the blood insulin level decreases fatty acid synthesis due largely to a reduced rate of glucose influx into adipocytes.

Starvation causes a long term reduction in fatty acid synthesis. This results from a decline in the activities of ATP-citrate lyase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, acetyl-CoA carboxylase and fatty acid synthase, probably because of a fall in the circulating insulin level.

13.6 ELONGATION OF FATTY ACIDS

Microsomal chain elongation

The cytoplasmic *de novo* synthesis of fatty acids ends with the production of palmitic acid (C_{16}) in the liver and adipose tissue. Stearic (C_{18}) and other higher fatty acids, longer than palmitate, are formed from palmitate by enzymes of the *microsomal*

elongase or chain elongation system in the smooth endoplasmic reticulum. These enzymes elongate pre-existing C_{16} and higher fatty acids, both saturated and unsaturated, by two carbons at each step. Like fatty acid synthase, microsomal elongase system also uses malonyl-CoA and NADPH as the sources of respectively the C_2 units and the reducing equivalents (H^+ and e). The source of malonyl-CoA is the carboxybiotin-mediated carboxylation of acetyl-CoA in the cytoplasm by acetyl-CoA carboxylase at the cost of one high-energy bond of ATP (page 413). But unlike the synthase complex, enzymes of the elongase system exist as separate entities and not as a multienzyme complex. Nor is ACP involved in binding or carrying the acyl group during their action; instead, the chain elongation enzymes use acyl-CoA molecules directly as substrates. Moreover, the fresh C_2 units are added by these enzymes to the carboxyl end of pre-existing fatty acid chains instead of their ω -methyl end.

The reaction sequence for elongating palmitate (C_{16}) to stearate (C_{18}) is given below (Fig. 13.18).

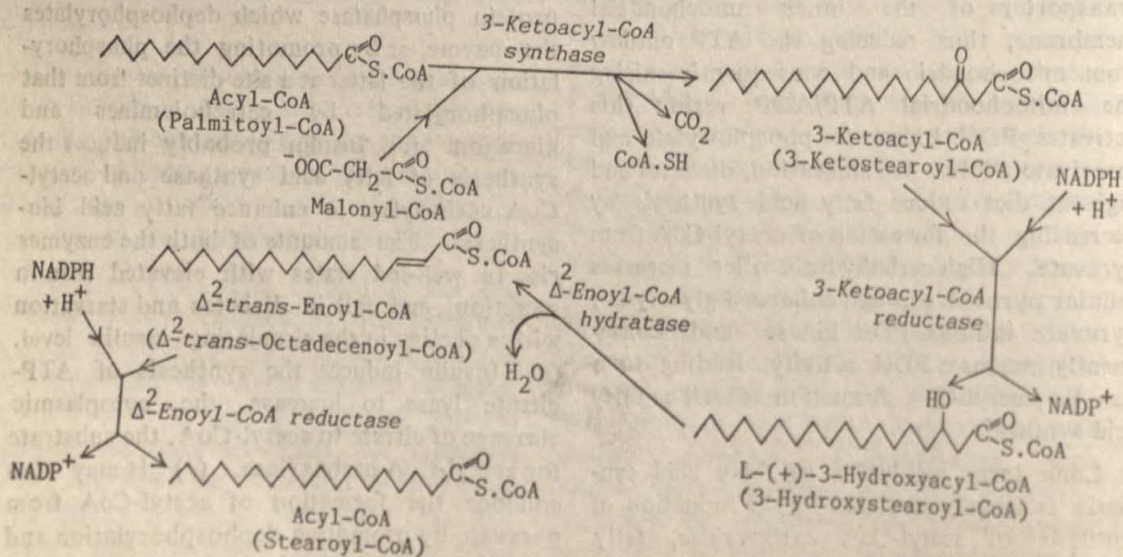


Fig. 13.18. Microsomal chain elongation.

(a) *3-Ketoacyl-CoA synthase* transfers the acyl (palmitoyl) group from acyl-CoA (palmitoyl-CoA) to the methylene-C of malonyl-CoA, releasing simultaneously the carboxyl-C of the latter as CO_2 . As in the case of fatty acid synthase, the carboxyl-C of malonate, originally derived from HCO_3^- by acetyl-CoA carboxylase action, does not find its way into the elongated fatty acid chain. The energy released by the cleavage of the bond between the methylene-C and the carboxyl-C of malonate, is used in forming the new C—C bond linking the methylene-C of malonate with the carbonyl-C of the palmitoyl group. A 3-ketoacyl-CoA (3-ketostearoyl-CoA in this case) is thus formed.

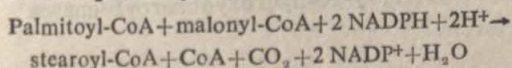
(b) *3-Ketoacyl-CoA reductase* next transfers reducing equivalents from NADPH to the C^3 -ketone group of 3-ketoacyl-CoA, reducing the latter to L(+)-3-hydroxyacyl-CoA, i.e., 3-hydroxystearoyl-CoA in the present case.

(c) Δ^2 -Enoyl-CoA hydratase then dehydrates 3-hydroxyacyl-CoA to Δ^2 -trans-enoyl-CoA (Δ^2 -trans-octadecenoyl-CoA in this case) by removing H and OH groups from C^2 and C^3 respectively.

(d) Δ^2 -Enoyl-CoA reductase transfers reducing equivalents from NADPH to C^2 and C^3 of Δ^2 -trans-enoyl-CoA, saturating its C^2 - C^3 double-bond and changing it to a new acyl-CoA which exceeds the previous acyl-CoA by two carbons. Starting from palmitoyl-CoA, stearoyl-CoA is produced as the new acyl-CoA in this step.

This cycle of steps (a) to (d) may be repeated a number of times, elongating the acyl chain further by two carbons in each cycle.

Following is the stoichiometry of elongation of palmitoyl-CoA to stearoyl-CoA :



The elongation of the acyl group by each C_2 unit costs one high-energy bond of ATP,

which is spent in the biotin-dependent carboxylation of acetyl-CoA to malonyl-CoA before the latter may be used in the process.

Mitochondrial chain elongation

In anaerobic conditions, saturated and unsaturated fatty acids (C_{12} to C_{18}) may be elongated upto C_{24} fatty acids by a mitochondrial elongase system. The latter uses acetyl-CoA directly for incorporating C_2 units

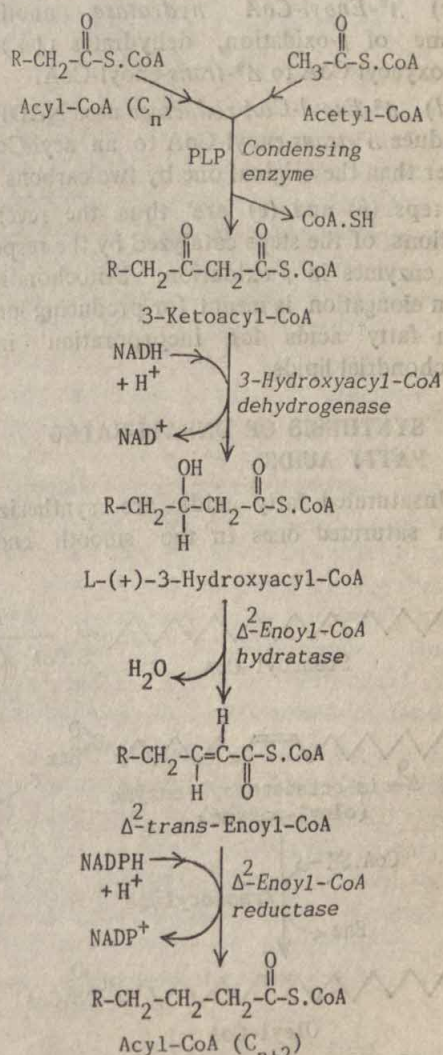


Fig. 13.19. Mitochondrial chain elongation.

into pre-existing acyl-CoA molecules; neither ACP nor malonyl-CoA is used in this process.

(a) A PLP-dependent *condensing enzyme* condenses an acetyl-CoA molecule with the pre-existing acyl-CoA to give free coenzyme A and a 3-ketoacyl-CoA molecule (Fig. 13.19).

(b) *3-Hydroxyacyl-CoA dehydrogenase*, an enzyme of the β -oxidation pathway, transfers reducing equivalents (H^+ and e^-) from NADH to the C^3 of 3-ketoacyl-CoA, changing the latter to L(+)-3-hydroxyacyl-CoA.

(c) Δ^2 -Enoyl-CoA hydratase, another enzyme of β -oxidation, dehydrates L(+)-3-hydroxyacyl-CoA to Δ^2 -trans-enoyl-CoA.

(d) Δ^2 -Enoyl-CoA reductase uses NADPH to reduce Δ^2 -trans-enoyl-CoA to an acyl-CoA longer than the original one by two carbons.

Steps (b) and (c) are thus the reverse reactions of the steps catalyzed by the respective enzymes in β -oxidation. Mitochondrial chain elongation is meant for producing long-chain fatty acids for incorporation into mitochondrial lipids.

13.7 SYNTHESIS OF UNSATURATED FATTY ACIDS

Unsaturated fatty acids are synthesized from saturated ones in the smooth endo-

plasmic reticulum by *microsomal desaturase systems*. Each of the latter is a multienzyme system and introduces a double-bond at a specific position in the fatty acid chain.

Synthesis of monounsaturated fatty acids

A *microsomal Δ^9 desaturase* system of liver and adipose tissue introduces a Δ^9 double-bond (C^9-C^{10} double-bond) in a saturated fatty acid molecule (e.g., stearic acid) to change the latter to the corresponding Δ^9 -cis-monoenoic acid (e.g., oleic acid). The Δ^9 desaturase has several enzymic components. They act in the following sequence in converting stearic acid to oleic acid.

(a) *Acyltransferase* (transacylase) transfers the stearyl group from stearyl-CoA to the active site of the monooxygenase component of the Δ^9 desaturase, producing a stearyl-enzyme complex (Fig. 13.20).

(b) A *cytochrome b_5 monooxygenase* hydroxylates the C^{10} of the enzyme-bound stearyl group with the help of molecular O_2 and either NADH or NADPH as the electron-donor. It is this monooxygenase (10-mono-oxygenase) which is responsible for the specific position (Δ^9 in this case) of the double-bond in the final product. The monooxygenase has 3 enzymic components, viz., a flavoprotein

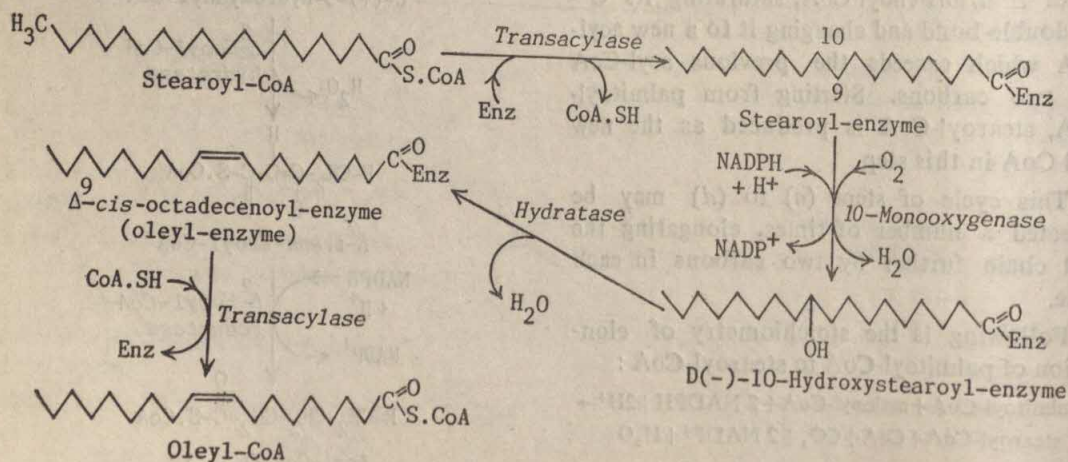


Fig. 13.20. Synthesis of oleyl-CoA from stearyl-CoA by microsomal Δ^9 desaturase system.

acting as the *NAD(P)H-cytochrome b_5 reductase*, an iron-sulfur protein and *cytochrome b_5* . Electrons are transferred to molecular O_2 from NADH or NADPH through successively the flavin prosthetic group of the reductase, the iron-sulfur centre and cytochrome b_5 . This reduces one oxygen atom of O_2 to H_2O while the other oxygen atom of O_2 is incorporated to the C^{10} of the stearyl group, changing the latter to the 10-hydroxystearoyl group.

(c) *Hydratase* component of the Δ^9 desaturase then dehydrates the enzyme-bound 10-hydroxystearoyl group into an oleyl group.

(d) *Acyltransferase* finally transfers the oleyl group from the desaturase to coenzyme A to produce oleyl-CoA.

Palmitoyl-CoA is similarly changed by Δ^9 desaturase to palmitoleyl-CoA with a Δ^9 double-bond.

Synthesis of polyenoic fatty acids

In hepatocytes and adipocytes, microsomal desaturases distinct from the Δ^9 desaturase incorporate one or more double-bonds at specific positions in Δ^9 -monoenoic acids to change them to the corresponding polyenoic acids. Microsomal Δ^6 desaturase, Δ^5 desaturase and Δ^4 desaturase systems incorporate double-bonds respectively at C^6-C^7 , C^5-C^6 and C^4-C^5 positions. Their modes of action resemble that of the Δ^9 desaturase. The monooxygenase component of each desaturase determines the specific position of the newly incorporated double-bond by hydroxylating a specific carbon of the fatty acid (e.g., C^7 , C^6 and C^5 in cases of respectively Δ^6 desaturase, Δ^5 desaturase and Δ^4 desaturase). The Δ^9 desaturase acts first on a saturated fatty acid to change it to a Δ^9 -*cis*-monoenoic acid; this may be followed by the incorporation of additional double-bonds in the monoenoic acid by the other desaturases. However, mammalian desaturases can incorporate double-bonds only on the carboxyl

terminal side and not on the methyl (ω) terminal side of the pre-existing double-bond. Moreover, two consecutive double-bonds are always separated from each other by a methylene group. Besides, the double-bonds incorporated by these desaturases are in *cis* conformation.

Different sequences of actions by microsomal elongase and desaturases convert oleic acid (Δ^9 -*cis*-octadecenoic acid) to ω -9 polyenoic acids of diverse chain-lengths and different numbers of double-bonds.

Essential fatty acids:

Linoleic ($\Delta^{9,12}$ -*cis*-octadecadienoic), linolenic ($\Delta^{9,12,15}$ -*cis*-octadecatrienoic) and arachidonic ($\Delta^{8,11,14}$ -*cis*-eicosatetraenoic) acids cannot be synthesized in the mammalian body and must be supplied with the diet, because their synthesis would need the introduction of double-bonds between the Δ^9 double-bond and the ω -methyl group, for which the mammalian tissues possess no desaturase. These three polyenoic acids are, therefore, called *essential fatty acids* (EFA). Linoleate (Fig. 2.1) occurs in the oils of corn, peanut, soyabean, cottonseed, safflower, sunflower and sesame, but is poor in coconut oil, olive oil and butter. Linolenate is present in much smaller amounts in soyabean oil while arachidonate occurs in small amounts in muscle fats and liver lipids. However, arachidonate may be synthesized in the mammalian body from linoleate of food.

Rats, made deficient in EFA by a fat-free diet, suffer from scaly dermatitis, thickening of skin, loss of hair, retarded growth, reproductive failure, fatty liver, urinary tract lesions, impaired stress resistance, necrosis of tail and decreased tissue prostaglandin. Feeding of linolenate stimulates the growth rate, but fails to cure skin lesions; linoleate cures both.

Dietary deficiency of EFA produces *phrynodema* in man. The disease is

characterized by rough, thickened and horny skin with keratinized papules, particularly on trunk, arms, buttocks and thighs. There also exist some relations between a dietary deficiency of EFA and eczematous lesions, arterial degeneration and fatty liver in man. EFA administration may lower the serum cholesterol in human adults and ameliorate eczema in children on skimmed milk diet.

Synthesis of arachidonate from linoleate: Linoleate is converted to arachidonate in the mammalian liver by the successive actions of microsomal desaturase and elongase systems.

(a) Microsomal Δ^6 desaturase system incorporates a Δ^6 double-bond in linoleyl-CoA to produce γ -linolenyl-CoA ($\Delta^{6,9,12}$ -*cis*-octadecatrienoyl-CoA) with the help of molecular O_2 , NADH or NADPH, FAD, iron-sulfur protein and cytochrome b_5 (Fig. 13.21). The mode of action resembles that of Δ^9 desaturase described earlier.

(b) γ -Linolenyl-CoA receives a C_2 unit from malonyl-CoA to form dihomogamma-linolenyl-CoA ($\Delta^{8,11,14}$ -*cis*-eicosatrienoyl-CoA) with the help of NADPH and the microsomal elongase system (see page 418).

(c) Microsomal Δ^5 desaturase system next introduces a Δ^5 double-bond into dihomogamma-linolenyl-CoA in the same manner as the Δ^6 desaturase, forming arachidonyl-CoA.

This process may serve as an example of how pre-existing fatty acids can be changed to new ones by a suitable combination of desaturase and elongase actions.

Functions of essential fatty acids:

(a) Essential fatty acids serve as precursors of prostaglandins (PG), thromboxanes (TX) and leukotrienes (LT). Prostaglandins and thromboxanes of series 1 (e.g., PGE_1 , PGF_1 and TXA_1) and leukotrienes of series 3 (e.g., LTA_3 and LTC_3) are synthesized from dihomogamma-linolenate derived from linoleic acid. Arachidonic acid gives rise to PG_2 and TX_2 series (e.g., PGE_2 , $PGF_{2\alpha}$ and TXA_2) and

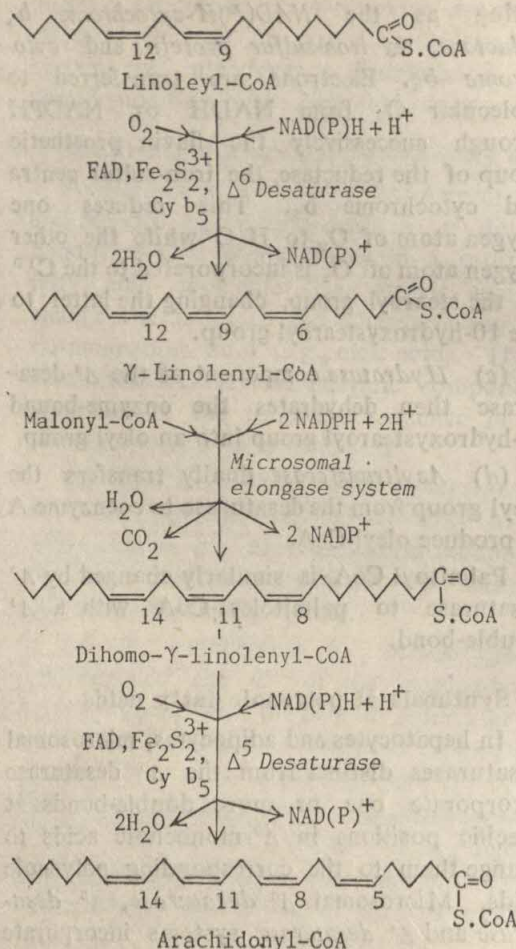


Fig. 13.21. Synthesis of arachidonyl-CoA from linoleyl-CoA.

LT_4 series (e.g., LTA_4). Timnodonic acid, formed from linolenate, gives rise to PG_3 and TX_3 series (e.g., PGD_3 and TXA_3) and LT_5 series (e.g., LTA_5). (b) Essential fatty acids are used in phospholipid synthesis and are incorporated particularly as the 2-acyl (β -acyl) group in phosphoglycerides. (c) As constituents of phosphoglycerides, they are essential for the structural integrity of lipoprotein membranes of cells. (d) They promote fat mobilization and prevent fatty liver by participating in phospholipid formation. (e) They help to lower the serum cholesterol, particularly

the serum LDL-cholesterol. (f) Successive actions of microsomal elongase and desaturases synthesize various polyenoic fatty acids of ω -6 and ω -3 series from respectively linoleic and linolenic acids.

13.8 PROSTAGLANDINS, THROMBOXANES AND LEUKOTRIENES

Prostaglandins (PG), prostacyclin (PGI_2), thromboxanes (TX) and leukotrienes (LT) are derivatives of C_{20} polyunsaturated (eicosapolyenoic) fatty acids. PGs as well as PGI_2 bear a cyclopentane (C_5) ring at the middle of their hydrocarbon chains (Fig. 2.31). TXs bear an oxane ring instead of the cyclopentane ring. LTs possess no ring, but have three characteristic conjugated double-bonds.

These compounds belong to different series, indicated by subscript numbers showing the number of double-bonds in their linear hydrocarbon chains; e.g., PG_1 , PG_2 and PG_3 series; TX_1 , TX_2 and TX_3 series; LT_3 , LT_4 and LT_5 series. Different compounds of each series are characterized by the substituent groups on the cyclopentane or oxane ring or on the linear chain, and are designated by letters like A, B, C, D, E and F; e.g., PGA_1 , PGE_2 , TXA_2 , LTA_4 , etc.

Synthesis

Cyclo-oxygenase pathway:

Prostaglandins, prostacyclin and thromboxanes are synthesized from different eicosapolyenoic acids by the *cyclo-oxygenase pathway*. About 1 mg of prostaglandins is synthesized in 24 hours in the renal medulla, renal calyces, seminal vesicles and lungs in particular and also in the liver, brain, uterus, heart, adipose tissue, thymus, renal cortex, gastrointestinal tract and platelets. Vascular endothelium, heart and kidneys are the major sites for prostacyclin synthesis. Thromboxanes are mainly synthesized in platelets, polymorphonuclear leucocytes, lungs and brain.

(a) The first and *rate-limiting* enzyme of the pathway is a phospholipase using Ca^{++} as a cofactor. The phospholipase releases an eicosapolyenoic acid from membrane phospholipids which generally contain such polyenoic acids esterified to the C^2 of glycerol.

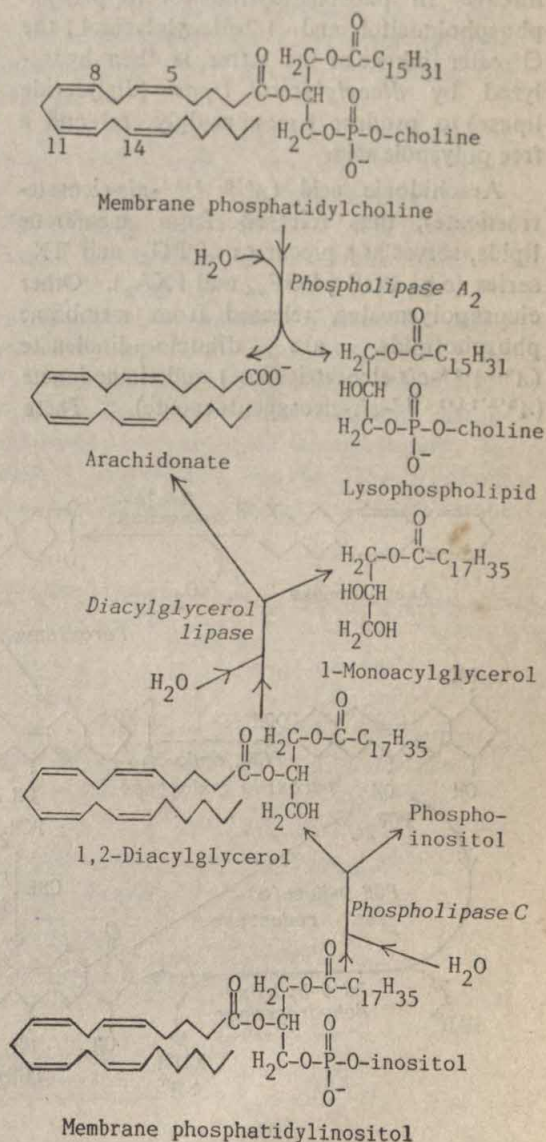


Fig. 13.22. Release of free arachidonate from membrane phospholipids.

In many tissues, *phospholipase A₂* hydrolyzes the C²-ester linkage of membrane glycerophospholipids to produce a lysophospholipid and a free polyenoic acid (Fig. 13.22). Alternatively, at least in platelets, *phospholipase C* hydrolyzes the glycerophosphate ester linkage in phosphatidylinositol to produce phosphoinositol and 1,2-diacylglycerol; the C²-ester linkage of the latter is then hydrolyzed by *diacylglycerol lipase* (diglyceride lipase) to produce 1-monoacylglycerol and a free polyenoic acid.

Arachidonic acid ($\Delta^{5,8,11,14}$ -*cis*-eicosatetraenoate), thus released from membrane lipids, serves as a precursor of PG₂ and TX₂ series (e.g., PGE₂, PGF_{2 α} and TXA₂). Other eicosapolyenoates, released from membrane phospholipids, are dihomog γ -linolenate ($\Delta^{8,11,14}$ -*cis*-eicosatrienoate) and timnodonate ($\Delta^{5,8,11,14,17}$ -*cis*-eicosapentaenoate). These

give rise to compounds of respectively series 1 (e.g., PGE₁, PGF_{1 α} and TXA₁) and series 3 (e.g., PGE₃, PGF_{3 α} and TXA₃).

The rate of prostaglandin synthesis is largely controlled by factors regulating phospholipases. Adrenocorticoids and mepacrine inhibit the phospholipase to decrease prostaglandin synthesis; trauma, hypoxia, angiotensin and bradykinin enhance prostaglandin synthesis by activating phospholipases.

(b) On release from membrane phospholipids, arachidonate and other eicosapolyenoates are oxidatively cyclized into respective primary prostaglandins (viz., PGE₂, PGF_{2 α} , PGE₁, PGF_{1 α} , PGE₃ and PGF_{3 α}) by *prostaglandin synthase*. The latter is a multi-enzyme complex of the microsomal (endoplasmic reticulum) membrane. One of its components, called *fatty acid cyclo-oxygenase* or *PG endoperoxide synthase*, is a dimeric

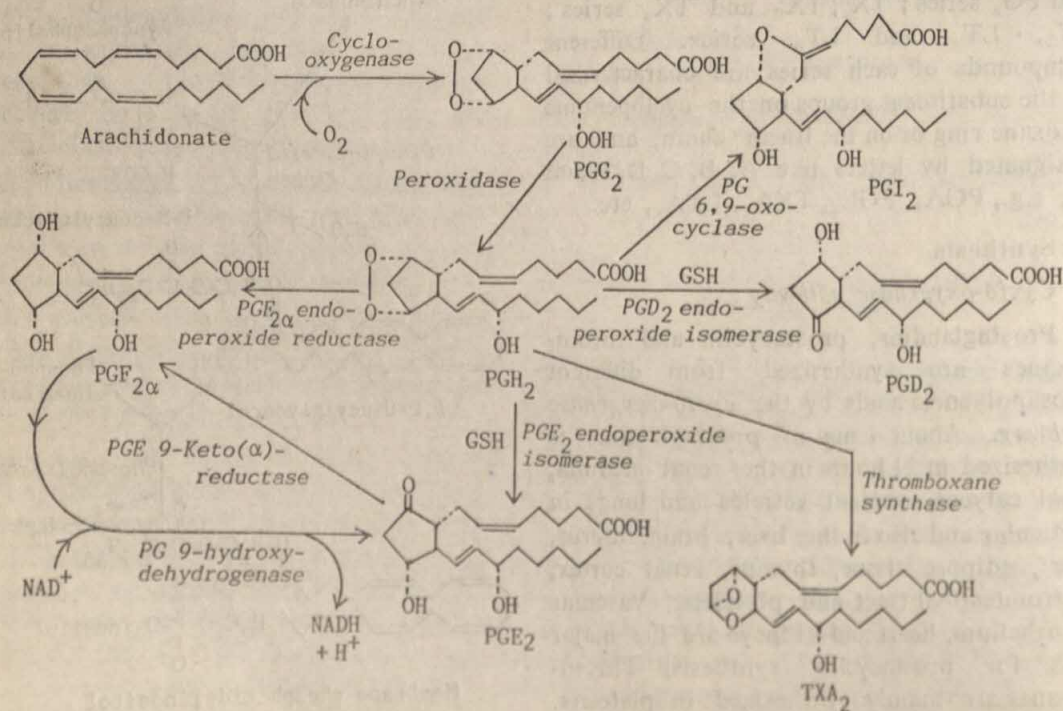


Fig. 13.23. The cyclo-oxygenase pathway for the synthesis of series 2 prostaglandins and thromboxanes from arachidonate. GSH: glutathione.

protein with both non-heme iron and heme in its prosthetic group. This endoperoxide synthase acts first as a cyclo-oxygenase to catalyze the oxygenation and cyclization of the eicosapentaenoic acids into unstable endoperoxides; arachidonate is thus changed to PGG_2 . The endoperoxide synthase next acts as a peroxidase to change PGG_2 to PGH_2 , another unstable cyclic endoperoxide (Fig. 13.23). Aspirin, indomethacin and some other non-steroid anti-inflammatory agents prevent the conversion of arachidonate to PGG_2 by affecting a hydrophobic site of the cyclo-oxygenase component. Catecholamines enhance prostaglandin synthesis by changing inactive cyclo-oxygenase to its active form.

(c) Microsomal prostaglandin endoperoxide isomerases isomerize PGH_2 to either PGE_2 or PGD_2 with the help of glutathione.

(d) Alternatively, prostaglandin endoperoxide reductase reduces PGH_2 to $\text{PGF}_{2\alpha}$. In brain and kidneys, however, *PGE 9-keto(α)-reductase* reduces PGE_2 directly to $\text{PGF}_{2\alpha}$.

The latter may be oxidized back to PGE_2 by *PG 9-hydroxydehydrogenase* and NAD^+ .

(e) *Dehydratases* may change PGEs to PGBs and PGAs while the latter may be isomerized to corresponding PGCs by *PGA isomerase*.

(f) *Prostacyclin synthase* or *prostaglandin 6,9-oxocyclase* of vascular endothelium cyclizes PGH_2 further into prostacyclin (PGI_2).

(g) Particularly in platelets, polymorphs, lungs and brain, *thromboxane synthase* converts PGH_2 to the thromboxane TXA_2 . Dipyridamole and imidazole blocks thromboxane synthesis by inhibiting this enzyme.

In the same way as the conversion of arachidonate to series 2 compounds, other eicosapolyenoates are changed to series 1 and 3 compounds like PGA_1 , $\text{PGF}_{3\alpha}$ and PGE_3 . Arachidonate is the most frequent eicosapolyenoate in membrane phospholipids; so, series 2 compounds are generally more abundant than others in most tissues. Each tissue

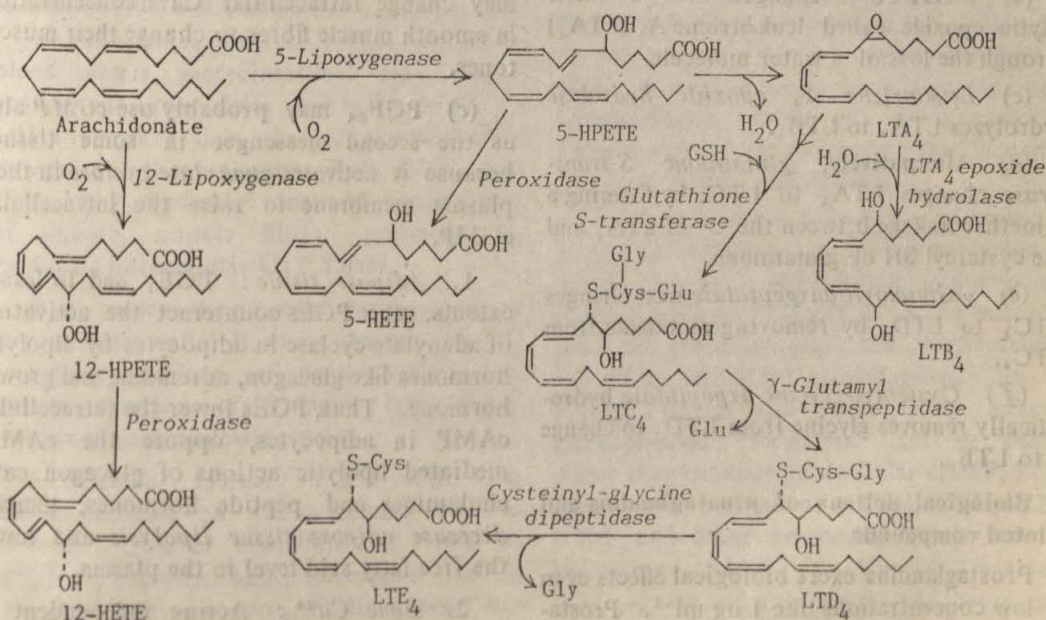


Fig. 13.24. The lipoxygenase pathway for leukotriene synthesis from arachidonate.

predominates in a specific enzyme for metabolizing PG endoperoxides. This determines whether prostaglandins, thromboxanes or prostacyclin will be the predominant product of the cyclo-oxygenase pathway in that tissue.

Catecholamines, vasopressin, kinins and angiotensin stimulate prostaglandin synthase activity to enhance the rate of this pathway.

Lipoxygenase pathway :

Leukotrienes are synthesized from eicosapolyenoates by this pathway in mast cells, basophils, macrophages, neutrophil leucocytes, platelets and lungs.

(a) Cytoplasmic dioxygenases called *lipoxygenases* oxygenate arachidonate, released from membrane phospholipids, at specific positions to form specific hydroperoxyeicosatetraenoates (HPETE). For example, 5-*lipoxygenase* of neutrophils and 12-*lipoxygenase* of platelets oxygenate arachidonate at 5 and 12 positions respectively to produce 5-HPETE in neutrophils and 12-HPETE in platelets (Fig. 13.24).

(b) 5-HPETE is changed into a labile allylic epoxide called leukotriene A_4 (LTA_4) through the loss of a water molecule.

(c) *Leukotriene A_4 epoxide hydrolase* hydrolyzes LTA_4 to LTB_4 .

(d) Alternatively, *glutathione S-transferase* changes LTA_4 to LTC_4 by forming a thioether linkage between the C^6 of LTA_4 and the cysteinyl SH of glutathione.

(e) γ -*Glutamyltranspeptidase* next changes LTC_4 to LTD_4 by removing glutamate from LTC_4 .

(f) *Cysteinyl-glycine dipeptidase* hydrolytically removes glycine from LTD_4 to change it to LTE_4 .

Biological actions of prostaglandins and related compounds

Prostaglandins exert biological effects even at low concentrations like 1 ng ml^{-1} . Prostaglandins, thromboxanes and prostacyclin bind

with specific receptors on the plasma membrane of their target cells and thereby change the intracellular concentrations of different secondary messengers such as cAMP, Ca^{2+} and cGMP, which then mediate in the biological effects. PGEs, PGFs and PGD_2 appear to bind with specific membrane receptors distinct from each other, but PGI_2 may bind with PGE receptors of platelet membrane.

(a) At least some effects of prostaglandins and related compounds depend on their action in changing the intracellular cAMP level. Prostaglandins activate *adenylate cyclase* in the plasma membrane to raise the cAMP level in platelets, corpus luteum, anterior pituitary, thyroid, lungs and bones; PGEs are much more potent than PGFs in activating *adenylate cyclase*. On the contrary, PGEs can lower the cAMP level in adipocytes and renal tubule cells. TXA_2 inhibits *adenylate cyclase* in platelets to reduce the platelet cAMP level.

(b) TXA_2 as well as PGFs may use Ca^{2+} as a second messenger in some tissues; they may change intracellular Ca^{2+} concentration in smooth muscle fibres to change their muscle tones.

(c) $PGF_{2\alpha}$ may probably use cGMP also as the second messenger in some tissues because it activates *guanylate cyclase* in their plasma membrane to raise the intracellular cGMP.

1. *Adipose tissue*: PGE_1 and to lesser extents, other PGEs counteract the activation of *adenylate cyclase* in adipocytes by lipolytic hormones like glucagon, adrenaline and growth hormone. Thus, PGEs lower the intracellular cAMP in adipocytes, oppose the cAMP-mediated lipolytic actions of glucagon, catecholamines and peptide hormones, thereby decrease adipose tissue lipolysis and lower the free fatty acid level in the plasma.

2. *Bone Ca^{2+}* : Acting independent of parathormone, PGE_1 and PGE_2 increase the

number and osteolytic activity of osteoclasts. Consequently, PGEs enhance the mobilization of Ca^{2+} from bones.

3. *Gastrointestinal secretions*: PGEs as well as PGI_2 inhibit gastrin secretion from the pyloric mucosa and consequently decrease gastric acid secretion. This action may be utilized in treating gastric ulcers. PGEs stimulate the secretion of pancreatic juice and intestinal mucus.

4. *Smooth muscles*: Both PGEs and PGFs stimulate contractions of uterine and gastrointestinal smooth muscles. So, they have therapeutic importance in inducing labor and terminating pregnancy; but a simultaneous increase in gastrointestinal motility, intestinal cramps and diarrhoea may pose a problem. $PGF_{2\alpha}$ and other PGFs contract smooth muscles on arterioles, trachea, bronchi and bronchioles to constrict them; $PGF_{2\alpha}$ may produce severe bronchospasms. But PGEs like PGE_1 and PGE_2 relax smooth muscles on arterioles, bronchi and bronchioles to dilate them; PGEs may, therefore, be used against asthmatic asphyxia.

Thromboxanes contract smooth muscles on blood vessels, gastrointestinal tract, uterus, bronchi and bronchioles; but their constrictor action is weaker than that of prostaglandins in some of these tissues. Both $PGF_{2\alpha}$ and TXA_2 may stimulate tone and contractions of smooth muscle fibres, probably by enhancing intracellular Ca^{2+} fluxes.

PGI_2 reduces the smooth muscle tone on the vascular wall. It also counteracts the constrictor actions of prostaglandins on smooth muscles of bronchi, bronchioles, uterus and gastrointestinal tract.

5. *Vascular system*: PGEs relax the smooth muscles on the arteriolar wall to produce a general vasodilatation, a fall in peripheral resistance and a consequent fall in arterial blood pressure. PGEs oppose the vasoconstrictor actions of angiotensin and

noradrenaline. They also increase the venous return to the heart to enhance the cardiac output. PGFs, on the contrary, contract the vascular smooth muscles, producing vasoconstriction and a rise in peripheral resistance.

PGI_2 exerts a stronger vasodilator effect than PGEs. Thromboxanes produce vasoconstriction.

6. *Kidneys*: (a) PGEs as well as PGI_2 increase the renal blood flow by causing renal vasodilatation. They thus help to enhance the glomerular filtration rate (GFR). (b) PGE_2 decreases the cAMP level in renal tubule cells and opposes the cAMP-mediated action of vasopressin on water reabsorption in tubules. Thus, PGE_2 reduces the reabsorption of water in distal tubules and collecting ducts of kidney and consequently makes the urine dilute and hypotonic. (c) PGEs enhance the urine volume by increasing the GFR and by reducing the vasopressin-induced reabsorption of water. Inhibitors of PGE synthesis may be used in treating diabetes insipidus resulting from vasopressin deficiency. (d) PGEs and PGAs decrease the tubular reabsorption of Na^+ and increase the urinary Na^+ elimination (natriuretic effect). (e) PGEs as well as PGI_2 stimulate renin secretion from renal juxtaglomerular cells. (f) PGEs also stimulate renal tissues to increase the formation of erythropoietin and kallikrein.

7. *Endocrines*: (a) PGEs increase the release of corticotropin, luteinizing hormone, growth hormone, thyrotropin, glucagon and corticosteroids. PGFs facilitate the release of pituitary gonadotropins and corticotropin. (b) PGEs stimulate steroidogenesis in the adrenal cortex and consequently enhance glucocorticoid secretion. (c) In tissues where they enhance intracellular cAMP, PGEs mimic the actions of ACTH, TSH, PTH, ICSH and other hormones which act by increasing cAMP. (d) On the contrary, PGEs oppose and decrease the cAMP-mediated actions of hormones in tissues where cAMP

level is reduced by PGEs; PGEs thus counteract the lipolytic actions of glucagon, adrenaline and peptide hormones in adipose tissue and the action of vasopressin in renal reabsorption of water. (e) PGEs also facilitate the *ovulatory action of LH* on ovarian follicles. PGF_{2α} causes atrophy of corpus luteum (*luteolysis*).

8. *Inflammation*: PGEs and PGD₂ released at the site of trauma or burn, produce vasodilatation, enhanced capillary permeability, cutaneous wheal formation and prolonged erythema. They also enhance the inflammatory effects of other agents like histamine. The anti-inflammatory action of aspirin results largely from its inhibitory effect on cyclo-oxygenase and a consequent decline in PGE synthesis. PGI₂ produces milder inflammatory effects and opposes platelet aggregation. TXA₂ and TXB₂ enhance platelet aggregation.

9. *Immunologic response*: PGEs secreted by macrophages may modulate or decrease the functions of B and T lymphocytes. They may also reduce the proliferation of lymphocytes in response to lymphocyte mitogenic factors.

10. *Platelet aggregation*: PGE₁ inhibits platelet aggregation by increasing the platelet cAMP level. PGE₂ inhibits platelet aggregation at higher concentrations, but enhances it at concentrations below 1 μM. PGD₂ also inhibits platelet aggregation, but by binding with membrane receptors distinct from those for PGEs.

TXA₂ formed in platelets decreases the platelet cAMP level by inhibiting adenylate cyclase, and consequently promotes platelet aggregation and thrombus formation strongly. TXA₂ also induces the release of serotonin, Ca²⁺, ADP, etc., from platelet granules.

PGI₂ is normally produced by vascular endothelium, binds with receptors for PGE on platelet membrane to activate adenylate

cyclase, consequently increases the platelet cAMP level and thereby strongly inhibits platelet aggregation. Injury to the vessel wall decreases PGI₂ formation and consequently reduces the anti-aggregatory action of PGI₂. Unopposed action of TXA₂ may then cause platelet aggregation and thrombus formation.

Biological actions of leukotrienes

Leukotrienes appear to act as mediators in anaphylaxis and inflammations.

1. *Slow-reacting substance of anaphylaxis (SRS-A)*: A mixture of LTC₄, LTD₄ and LTE₄ is released from mast cells during anaphylactic reactions. These leukotrienes act as SRS-A to cause sustained contractions of smooth muscles, particularly of bronchioles. They can also cause vasodilatation and increase vascular permeability (page 240).

2. *Chemotactic action*: LTB₄ stimulates chemotaxis and chemokinesis of neutrophils. It also increases the density of Fc receptors and C3b receptors on the plasma membrane of neutrophils and eosinophils for binding respectively IgG and C3b molecules. LTB₄ promotes a moderate release of lysosomal hydrolases from neutrophils.

Catabolism

Prostaglandins are rapidly catabolized in lungs, liver, brain and many other tissues

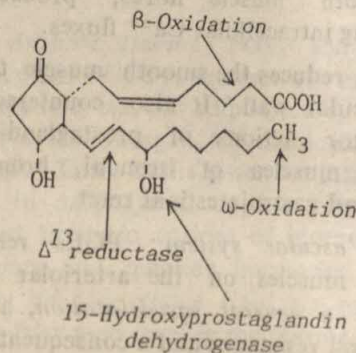


Fig 13.25. Catabolic changes at different sites of PGE₂.

(Fig. 13.25). 15-Hydroxyprostaglandins such as PGE_2 and PGF_2 are oxidized to the corresponding 15-ketoprostaglandins by 15-hydroxyprostaglandin dehydrogenases, using either NAD^+ or NADP^+ as the coenzyme. The $\Delta^{1,3}$ double-bond of 15-ketoprostaglandins is next reduced by prostaglandin $\Delta^{1,3}$ reductase to form the corresponding 15-keto-13,14-dihydroprostaglandins. These may undergo either β -oxidation to dinor and tetranor derivatives, or ω -oxidation to ω -hydroxy compounds and finally to dioic (dicarboxylic) acids.

13.9 TRIACYLGLYCEROL SYNTHESIS

Acylglycerols are mostly synthesized by enzymes of the smooth endoplasmic reticulum (ER) of liver, adipose tissue, lactating mammary glands, intestinal mucosa and

muscles. Smaller amounts are synthesized in mitochondria.

Acyl-CoA used in triacylglycerol synthesis is synthesized either from acetyl-CoA produced by carbohydrate metabolism, or from circulating free fatty acids produced by adipose tissue lipolysis. In the liver, the first is the major source in well-fed states while the second is the principal source during starvation, diabetes and high-fat intakes.

Glycerophosphate pathway

(a) In the liver and mammary glands, glycerol kinase uses ATP to phosphorylate glycerol to glycerol 3-phosphate (Fig. 13.26). But in the adipose tissue, intestinal mucosa and muscles, dihydroxyacetone phosphate from glycolysis is reduced to glycerol

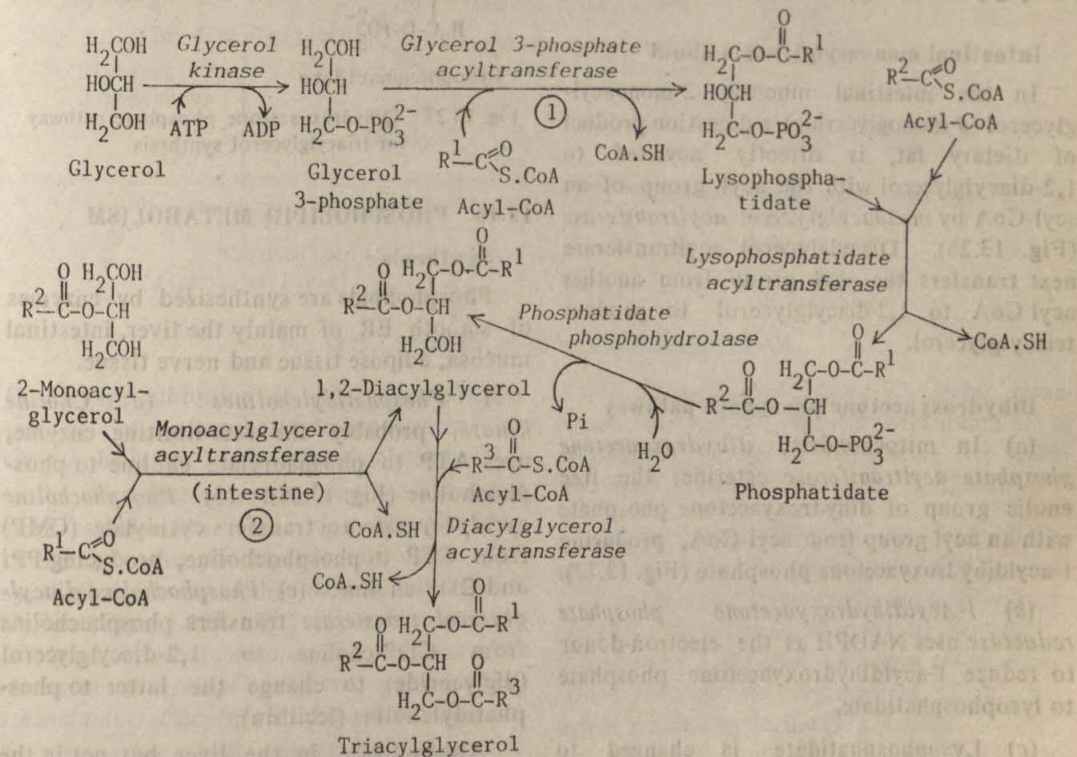


Fig. 13.26. Synthesis of triacylglycerols. (1) Glycerophosphate pathway. (2) Monoacylglycerol shunt.

3-phosphate by *glycerol 3-phosphate dehydrogenase* and NADH.

(b) *Glycerol 3-phosphate acyltransferase* transfers the acyl group from an acyl-CoA molecule to the C¹ of glycerol 3-phosphate to change the latter to lysophosphatidic acid.

(c) *Lysophosphatidate acyltransferase* transfers the acyl group from another acyl-CoA to the C² of lysophosphatidate, changing the latter to phosphatidic acid.

(d) *Phosphatidate phosphohydrolase*, occurring both on the ER membrane and in the cytosol, hydrolyzes phosphatidate to Pi and 1,2-diacylglycerol (diglyceride).

(e) *Diacylglycerol acyltransferase* transfers the acyl group from another acyl-CoA to the C³ of 1,2-diacylglycerol, changing it to triacylglycerol (triglyceride).

Intestinal monoacylglycerol shunt

In the intestinal mucosa, 2-monoacylglycerol (β -monoglyceride), a digestion product of dietary fat, is directly acylated to 1,2-diacylglycerol with the acyl group of an acyl-CoA by *monoacylglycerol acyltransferase* (Fig. 13.26). *Diacylglycerol acyltransferase* next transfers the acyl group from another acyl CoA to 1,2-diacylglycerol to produce triacylglycerol.

Dihydroxyacetone phosphate pathway

(a) In mitochondria, *dihydroxyacetone phosphate acyltransferase* esterifies the free enolic group of dihydroxyacetone phosphate with an acyl group from acyl-CoA, producing 1-acyldihydroxyacetone phosphate (Fig. 13.27).

(b) *1-Acyldihydroxyacetone phosphate reductase* uses NADPH as the electron-donor to reduce 1-acyldihydroxyacetone phosphate to lysophosphatidate.

(c) Lysophosphatidate is changed to triacylglycerol as in the glycerophosphate pathway.

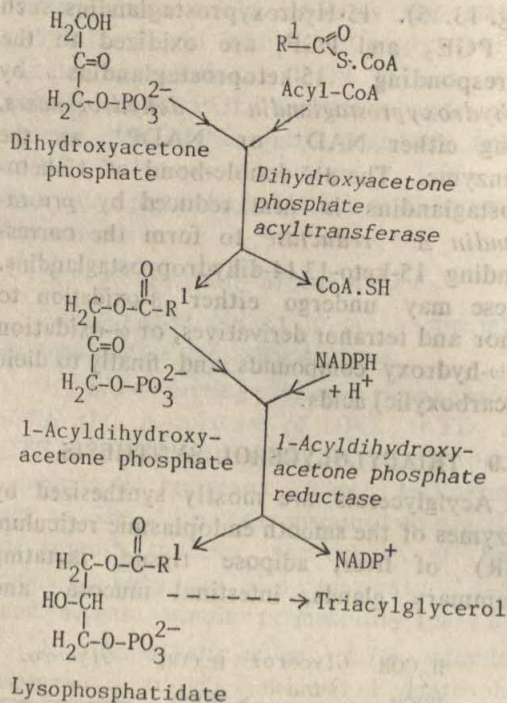


Fig. 13.27. Dihydroxyacetone phosphate pathway for triacylglycerol synthesis.

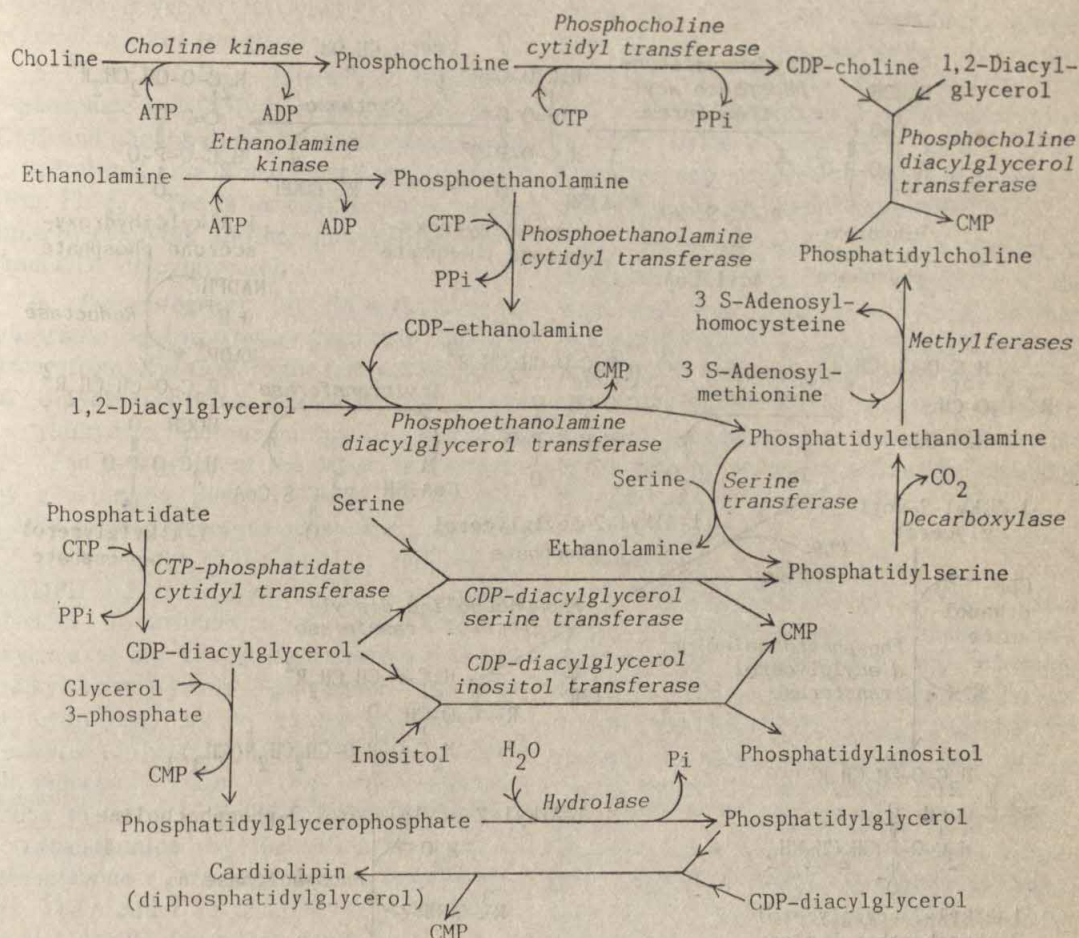
13.10 PHOSPHOLIPID METABOLISM

Synthesis

Phospholipids are synthesized by enzymes of smooth ER of mainly the liver, intestinal mucosa, adipose tissue and nerve tissue.

1 *Phosphatidylcholines*: (a) *Choline kinase*, probably the rate-limiting enzyme, uses ATP to phosphorylate choline to phosphocholine (Fig. 13.28). (b) *Phosphocholine cytidyl transferase* transfers cytidylate (CMP) from CTP to phosphocholine, producing PPi and CDP-choline. (c) *Phosphocholine diacylglycerol transferase* transfers phosphocholine from CDP-choline to 1,2-diacylglycerol (diglyceride) to change the latter to phosphatidylcholine (lecithin).

Alternatively, in the liver but not in the brain, *methyltransferases* transfer the labile methyl groups from three successive molecules of



S-adenosylmethionine to the ethanolamine residue of phosphatidylethanolamine to change the latter to phosphatidylcholine.

2. *Phosphatidylethanolamines*: Through steps similar to those for phosphatidylcholines, phosphatidylethanolamines are synthesized using ethanolamine instead of choline (Fig. 13.28). The successive steps are catalyzed by *ethanolamine kinase*, *phosphoethanolamine cytidyl transferase* and *phosphoethanolamine diacylglycerol transferase*.

Alternatively, phosphatidylserine may be changed to phosphatidylethanolamine by the decarboxylation of its serine (Fig. 13.28).

3. *Phosphatidylserines*: A serine transferase converts phosphatidylethanolamine to phosphatidylserine by replacing its ethanolamine residue with serine (Fig. 13.28).

Alternatively, *CTP-phosphatidate cytidyl transferase* transfers cytidylate (CMP) from CTP to phosphatidate, producing CDP-diacylglycerol; the latter is changed to phosphatidylserine by *CDP-diacylglycerol serine transferase* through the replacement of its cytidylate residue by serine (Fig. 13.28).

4. *Phosphatidylinositols*: These are produced by a reaction between inositol and

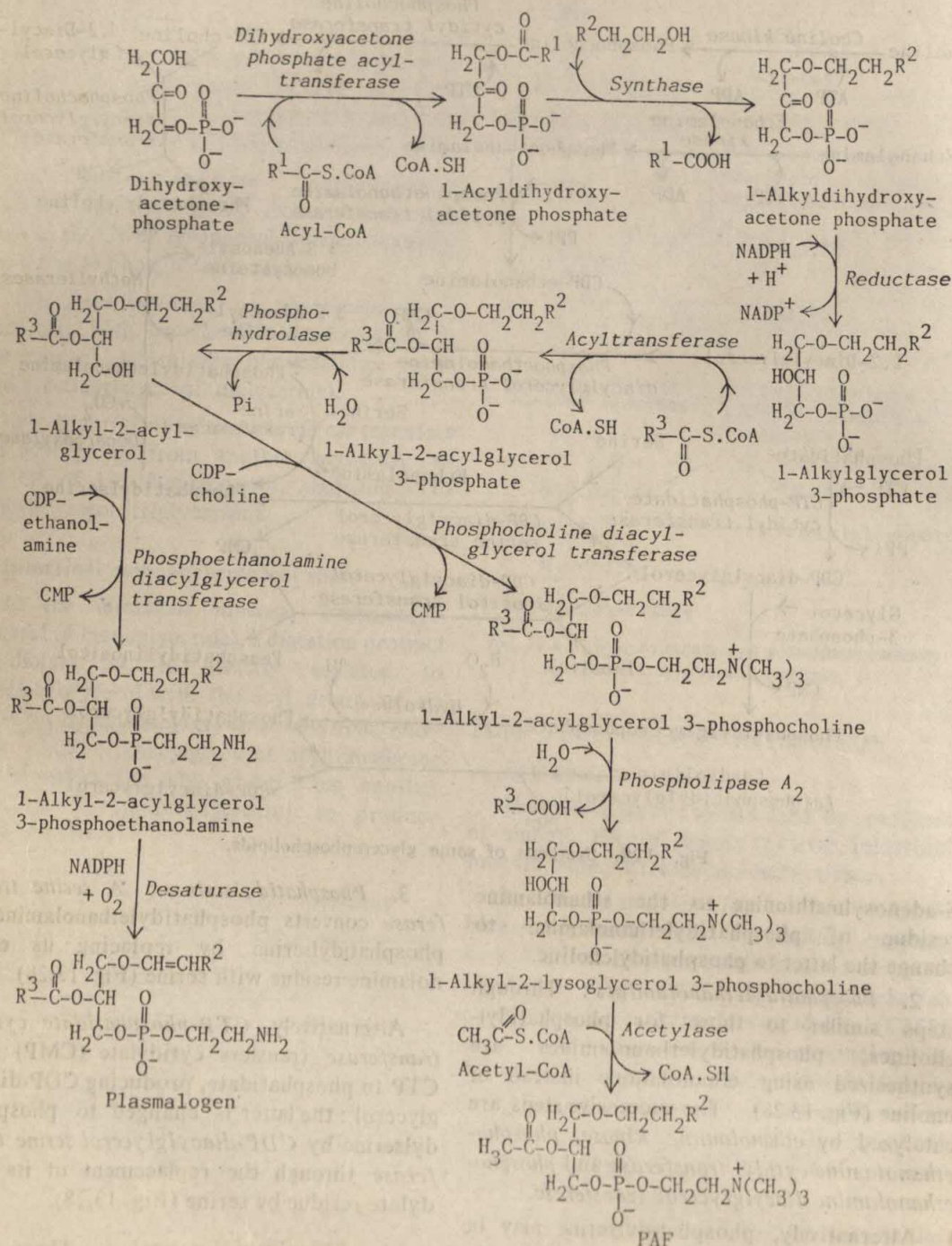


Fig. 13.29. Synthesis of plasmalogens and PAF.

CDP-diacylglycerol, catalyzed by *CDP-diacylglycerol inositol transferase* (Fig. 13.28).

5. *Cardiolipins*: Interaction of glycerol 3-phosphate and CDP-diacylglycerol produces CMP and phosphatidylglycerophosphate which is hydrolyzed into Pi and phosphatidylglycerol (Fig. 13.28). The latter changes into cardiolipin by receiving another phosphatidyl group from CDP-diacylglycerol.

6. *Plasmalogens*: (a) *Dihydroxyacetone phosphate acyltransferase* transfers an acyl group from acyl-CoA to the free enolic group of dihydroxyacetone phosphate to form 1-acyldihydroxyacetone phosphate (Fig. 13.29). (b) The acyl group of the latter is replaced by a saturated higher alcohol to produce 1-alkyldihydroxyacetone phosphate. (c) The C²-ketonyl group of the latter is reduced by NADPH and a *reductase* to produce 1-alkylglycerol 3-phosphate. (d) The latter is acylated at its C² by *acyltransferase* to give 1-alkyl-2-acylglycerol 3-phosphate. (e) This is hydrolyzed by a *phosphohydrolase* to 1-alkyl-2-acylglycerol. (f) The latter receives phosphoethanolamine from CDP-ethanolamine to form 1-alkyl-2-acylglycerol 3-phosphoethanolamine by the action of *phosphoethanolamine diacylglycerol transferase*. (g) The product is changed to plasmalogen by an NADPH-dependent *desaturase*.

7. *Platelet-activating factor or PAF*: (a) 1-Alkyl-2-acylglycerol, synthesized from dihydroxyacetone phosphate, receives phosphocholine from CDP-choline under the action of *phosphocholine diacylglycerol trans-*

ferase (Fig. 13.29). (b) The product, 1-alkyl-2-acylglycerol 3-phosphocholine, is hydrolyzed by *phospholipase A₂* into a fatty acid and 1-alkyl-2-lysoglycerol 3-phosphocholine. (c) The latter is acetylated at its C² by an *acetylase* and acetyl-CoA to yield 1-alkyl-2-acetyl-3-phosphocholine (PAF).

8. *Sphingomyelins*: (a) Sphingosine is synthesized from serine and palmitate in the brain and liver (page 382). (b) It is then N-acylated by *sphingosine acyltransferase* and acyl-CoA to form ceramide or N-acylsphingosine. (c) *Phosphocholine ceramide transferase* transfers phosphocholine from CDP-choline to the CH₂OH group of ceramide, changing the latter to sphingomyelin (Fig. 13.30).

Catabolism

1. *Glycerophospholipids*: These are largely catabolized by lysosomal and mitochondrial *phospholipases*. (a) *Phospholipase A₁* hydrolyzes the C¹-ester linkage of a glycerophospholipid (e.g., phosphatidylcholine) to yield a free fatty acid and a 2-acylglycerol 3-phospho-base like 2-acylglycerol 3-phosphocholine (Fig. 13.31). (b) *Phospholipase A₂* of liver, brain, adipocytes, intestinal mucosa and pancreas hydrolyzes the C²-ester linkage of a glycerophospholipid to liberate a free fatty acid and a lysophospholipid like lysophosphatidylcholine (lysolecithin) (Figs. 13.22 and 13.32). (c) *Phospholipase B* (lysophospholipase) of liver and intestinal mucosa hydrolyzes the C¹-ester linkage in a lysophospholipid (e.g., lysoleci-

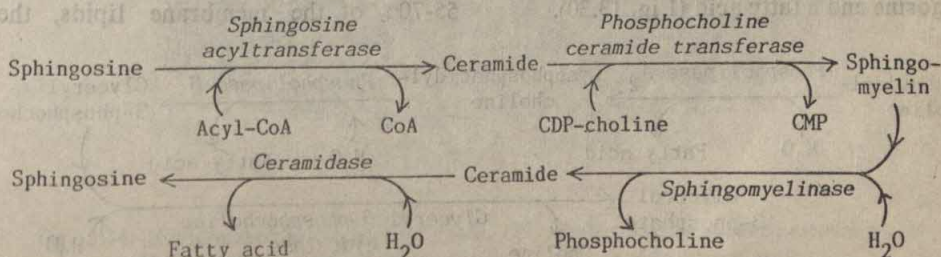


Fig. 13.30. Synthesis and catabolism of sphingomyelins.

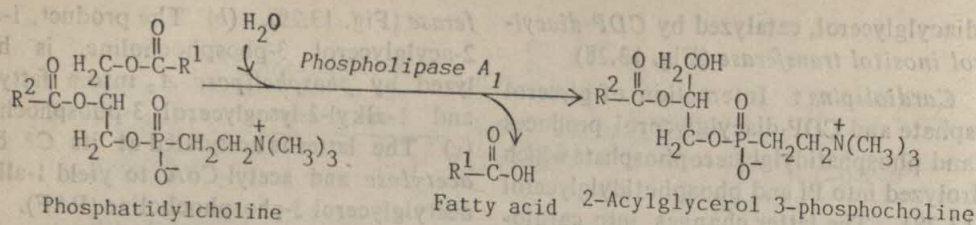


Fig. 13.31. Action of phospholipase A_1 .

thin) to give a fatty acid and a glycerophospho-base such as glyceryl 3-phosphocholine (Fig. 8.15). (d) *Phospholipase C* of brain and blood platelets hydrolyzes the glycerophosphate ester linkage in a glycerophospholipid to produce 1,2-diacylglycerol and a phospho-base like phosphocholine or phosphoinositol (Fig. 13.22).

Glycerol 3-phosphocholine hydrolase of liver is an esterase which hydrolyzes glyceryl 3-phosphocholine, produced by phospholipase B action, into choline and glycerol 3-phosphate (Fig. 13.32).

Lecithin-cholesterol acyltransferase of liver and blood plasma transfers a polyunsaturated acyl group from the C^2 of phosphatidylcholine to cholesterol, producing cholesteryl ester and lysophosphatidylcholine (Fig. 13.33). The latter may be further catabolized by phospholipase B and glyceryl 3-phosphocholine hydrolase.

2. *Sphingomyelins*: (a) *Sphingomyelinases* of brain, liver and spleen hydrolyze sphingomyelins to phosphocholine and ceramide. (b) The latter is hydrolyzed by *ceramidase* of brain and other tissues into sphingosine and a fatty acid (Fig. 13.30).

Farber's disease: This results from a genetic deficiency of ceramidase and is characterized by ceramide accumulation in brain, mental retardation, skin lesions and bone malformation.

Niemann-Pick disease: It results from a genetic deficiency of sphingomyelinase. Symptoms include accumulation of sphingomyelins in brain, liver and spleen, mental retardation, splenomegaly and hepatic enlargement.

Functions

Phospholipids are abundant in nerve tissue, liver, pancreas, blood, muscles and kidneys. White matter of brain contains about 8.5% phospholipid; grey matter contains only half that amount. Mitochondrial membranes contain significant amounts of cardiolipins and plasmalogens.

(a) According to the *Singer-Nicolson fluid-mosaic model*, cellular membranes are formed of a quasifluid lipid bilayer with many globular proteins embedded in it in the form of a discontinuous mosaic of particles (Fig. 13.34). Phosphoglycerides constitute 55-70% of the membrane lipids, the rest

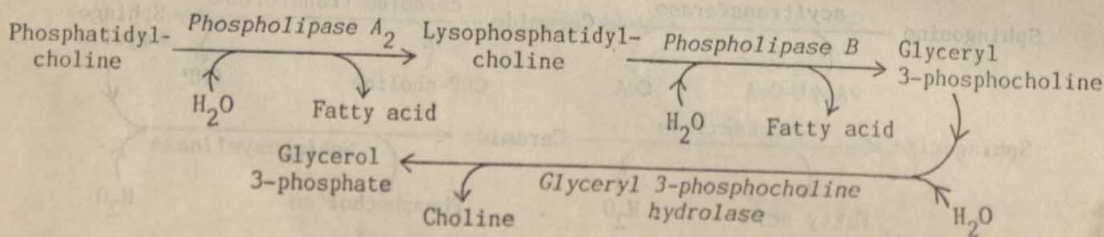


Fig. 13.32. Summary of phosphatidylcholine catabolism.

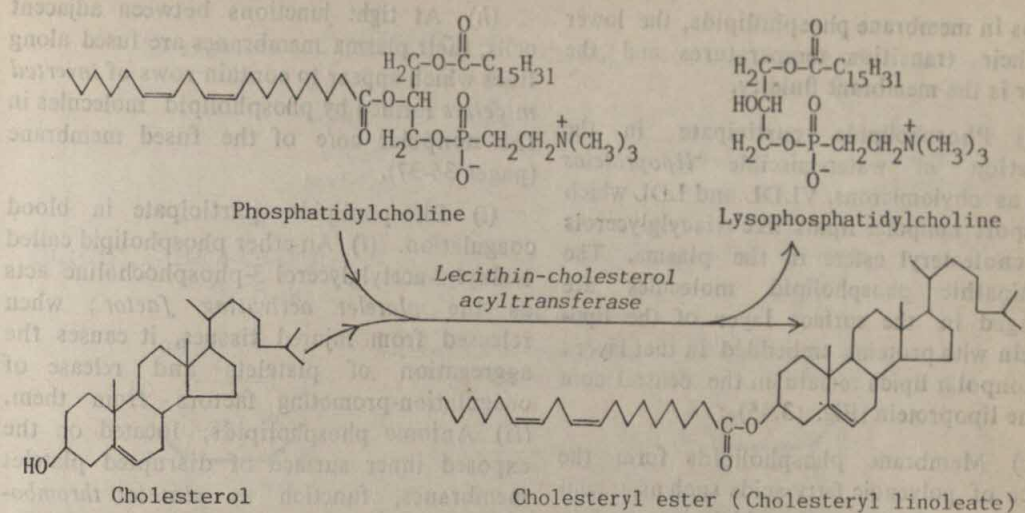


Fig.13.33. Action of lecithin-cholesterol acyltransferase.

consisting of sphingomyelins, cerebroside, gangliosides and cholesterol. The lipid bilayer consists of two monolayers of lipid molecules. The polar head-groups of the amphipathic phospholipid molecules of the two monolayers are oriented towards and cover respectively the outer and inner surfaces of the membrane; but the nonpolar tails of the phospholipid molecules of both monolayers are oriented towards the nonaqueous middle core of the membrane (page 37). The lipids are asymmetrically distributed in the membrane to contribute to its *inside-*

outside asymmetry—phosphatidylcholines, sphingomyelins and cholesterol occur mainly in the outer monolayer while phosphatidylserines and phosphatidylinositols occur mainly in the inner monolayer. The membrane has considerable *fluidity* and enables phospholipids and proteins to move freely in the lipid bilayer. This membrane fluidity depends largely on the *transition temperature* of its lipids, above which they remain in a more mobile liquid phase and below which, in a frozen or crystal phase. The higher the number of unsaturated acyl

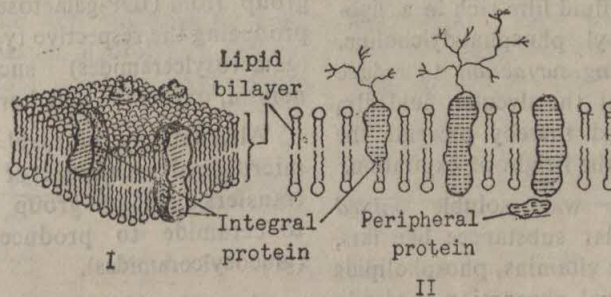


Fig.13.34. Fluid-mosaic model of membrane. I: A 3-dimensional view of membrane.

II: Branching oligosaccharide sidechains of integral proteins project on membrane surface.

groups in membrane phospholipids, the lower are their transition temperatures and the higher is the membrane fluidity.

(b) Phospholipids participate in the formation of water-miscible *lipoproteins* such as chylomicrons, VLDL and LDL which transport nonpolar lipids like triacylglycerols and cholesteryl esters in the plasma. The amphipathic phospholipid molecules are arranged in the surface layer of the lipoprotein with proteins embedded in that layer; the nonpolar lipids remain in the central core of the lipoprotein (Fig. 13.45).

(c) Membrane phospholipids form the source of polyenoic fatty acids such as arachidonate and timnodonate, which are used in the synthesis of *prostaglandins*, *thromboxanes* and *leukotrienes* (pages 423-426).

(d) Plasma phosphatidylcholines contribute polyenoic acyl groups for the *esterification of cholesterol* by lecithin-cholesterol acyltransferase. This helps to reduce the blood cholesterol level.

(e) Phospholipids of myelin sheaths insulate nerve fibres and thereby help in the *saltatory conduction* of nerve impulses in myelinated nerve fibres. Myelin sheath is particularly rich in phosphatidylcholines, phosphatidylethanolamines, sphingomyelins and plasmalogens.

(f) Inner surfaces of pulmonary alveoli are lined by a thin fluid film rich in a lipoprotein of dipalmitoyl phosphatidylcholine. The latter acts as a *lung surfactant* to reduce the surface tension of the alveolar fluid film during expiration and thereby prevents the collapse of alveoli at the height of expiration.

(g) By forming water-soluble *mixed micelles* with nonpolar substances like fats, sterols and fat-soluble vitamins, phospholipids help in their intestinal absorption as also in the excretion of cholesterol in bile (pages 36 and 198).

(h) At tight junctions between adjacent cells, their plasma membranes are fused along lines which appear to contain rows of *inverted micelles* formed by phospholipid molecules in the nonpolar core of the fused membrane (pages 36-37).

(i) Phospholipids participate in blood coagulation. (i) An ether phospholipid called 1-alkyl-2-acetyl-glycerol 3-phosphocholine acts as the *platelet activating factor*; when released from injured tissues, it causes the aggregation of platelets and release of coagulation-promoting factors from them. (ii) Anionic phospholipids, located on the exposed inner surface of disrupted platelet membranes, function as *platelet thromboplastic factors* in activating prothrombin to thrombin. (pages 221-222, 225).

13.11 GLYCOSPHINGOLIPID METABOLISM

Cerebrosides

Cerebrosides are synthesized by microsomal enzymes in the brain.

(a) *Acyltransferases* transfer C_{24} acyl groups (e.g., cerebronic, nervonic, oxynervonic and lignoceric acids) from the respective acyl-CoA molecules to the amino group of sphingosine to produce the corresponding types of ceramides (Fig. 13.35).

(b) *Galactosyl transferase* of brain microsomal membrane transfers a galactosyl group from UDP-galactose to each creamide, producing the respective types of cerebrosides (galactosylceramides) such as cerebron, nervon, oxynervon and kersin.

Alternatively, *glucosyl transferase* of the microsomal membrane of extraneural tissues transfers a glucosyl group from UDP-glucose to ceramide to produce glucocerebrosides (glucosylceramides).

A *cerebrosidase*, called galactosylceramide β -galactosidase, hydrolyzes cerebrosides into *galactose* and *ceramide* in the brain; *gluco-*

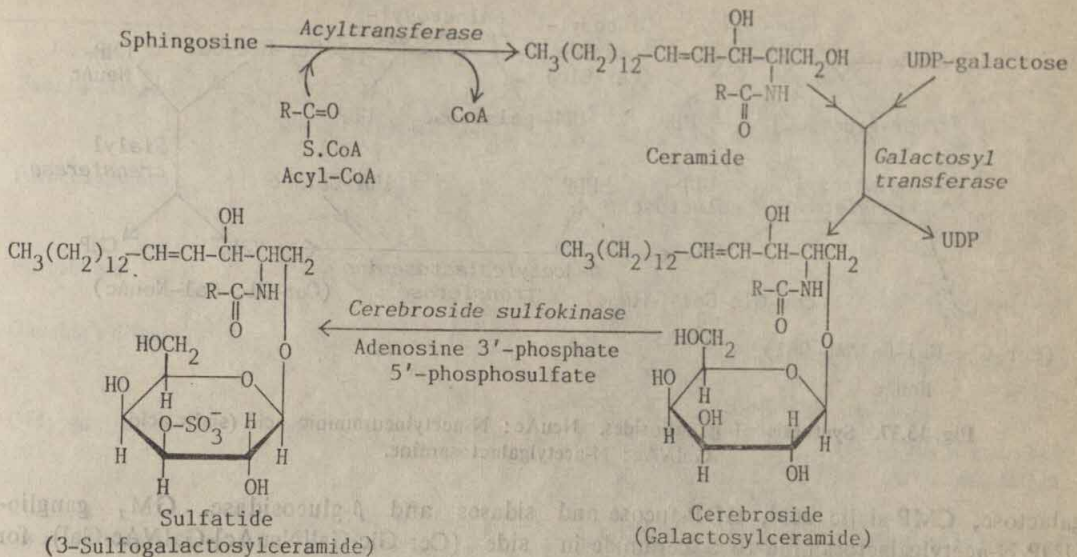


Fig. 13.35. Synthesis of cerebroside and sulfatides.

cerebrosidase similarly hydrolyzes glucocerebrosides into glucose and ceramide. *Ceramidase* of the brain then hydrolyzes ceramide into sphingosine and a C_{24} fatty acid (Fig. 13.36).

Sulfatides

Cerebroside sulfokinase of brain uses adenosine 3'-phosphate 5'-phosphosulfate as the sulfate-donor for the sulfation of the galactose residue of cerebroside, changing them to sulfatides like 3-sulfogalactosylceramide (Fig. 13.35).

Sulfatidases like aryl sulfatase of brain, liver and other tissues hydrolyze a sulfatide into inorganic sulfate and galactosylceramide

(cerebroside) (Fig. 13.36). The latter is hydrolyzed into galactose and ceramide by *galactosylceramide β -galactosidase*. *Ceramidase* finally hydrolyzes ceramide to sphingosine and a fatty acid.

Gangliosides

Different sugar group transferases such as glucosyl transferase, galactosyl transferase, glycolipid sialyl transferase, glycolipid fucosyl transferase and glycolipid N-acetylgalactosamine transferase occur in the Golgi membranes of many tissues including the brain and liver. They transfer specific sugar residues from the corresponding nucleotide-sugar complexes like UDP-glucose, UDP-

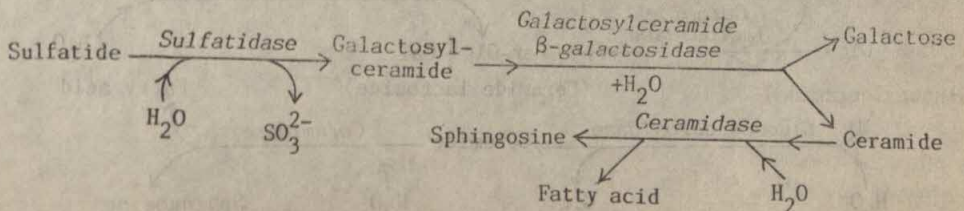


Fig. 13.36. Catabolism of cerebroside (galactosylceramide) and sulfatides.

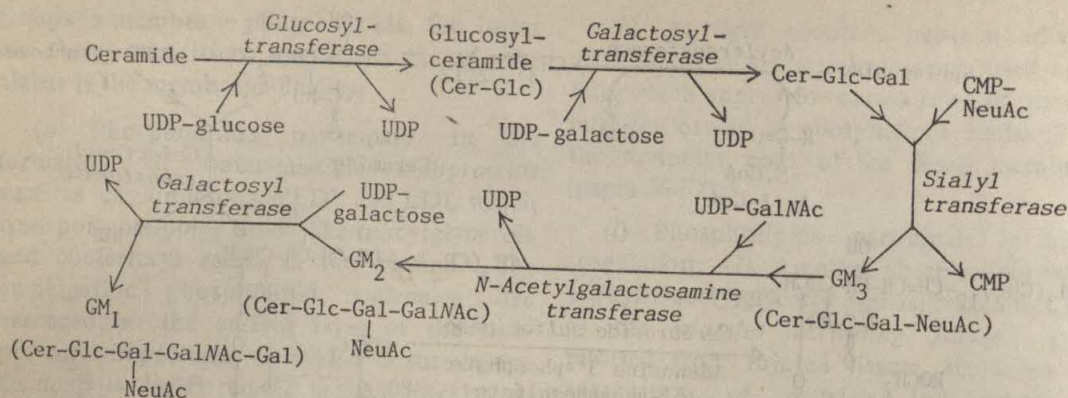


Fig. 13.37. Synthesis of gangliosides. NeuAc: N-acetylneuraminic acid (sialic acid). GalNAc: N-acetylgalactosamine.

galactose, CMP-sialic acid, GDP-fucose and UDP-N-acetylgalactosamine to a ceramide in specific sequences to produce different gangliosides (Fig. 13.37). Starting from the simplest GM₃ ganglioside (Cer-Glc-Gal-NeuAc), more complex GM₂, GM₁, GD₁ and other gangliosides may be synthesized by such progressive glycosylation.

Gangliosides are progressively hydrolyzed by lysosomal glycosyl hydrolases like GM₁- β -galactosidase, β -N-acetylhexosaminidase, N-acetylneuraminidase, α -fucosidase, galacto-

sidases and β -glucosidase. GM₁ ganglioside (Cer-Glc-Gal[NeuAc]-GalNAc-Gal), for example, is hydrolyzed by such enzymes to ceramide through GM₂ and GM₃ gangliosides, ceramide lactoside and glucosylceramide (Fig. 13.38). Ceramide is then hydrolyzed by ceramidase to fatty acid and sphingosine. Sphingolipidoses are inborn errors of sphingolipid metabolism due to genetic deficiencies of these hydrolases. These defects are inherited as autosomal recessive traits, except the maternally transmitted X-linked Fabry's disease (Table 13.1).

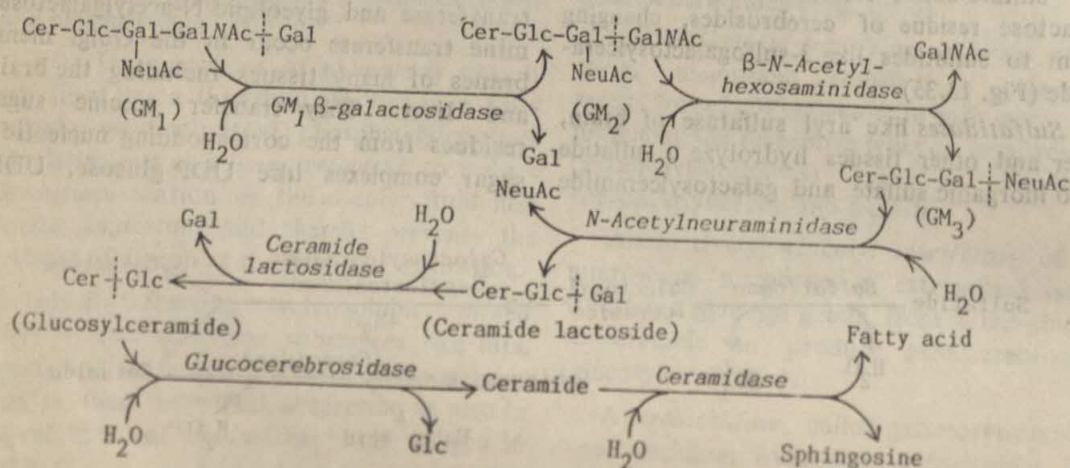


Fig. 13.38. Catabolism of gangliosides.

TABLE 13.1. *Some sphingolipidoses.*

Disease	Affected enzyme	Symptoms
Fabry's disease	Ceramide trihexosidase (α -galactosidase)	Accumulation of ceramide trihexoside in kidneys and intestine, renal and cardiac failures, skin eruptions, visual defects.
Farber's disease	Ceramidase	Accumulation of ceramides in brain, mental retardation, skin lesions, bone malformation, early death.
Fucosidosis	α -Fucosidase	Spasticity of muscles, dermatitis, cerebral degeneration.
Gaucher's disease	Glucocerebrosidase	Accumulation of glucocerebrosides in brain, bone marrow, spleen and liver; mental retardation, splenomegaly, loss of bone minerals.
GM ₁ gangliosidosis	GM ₁ - β -galactosidase	Accumulation of GM ₁ gangliosides in brain and viscera, bone malformation, mental retardation, hepatic enlargement.
Krabbe's leucodystrophy	Galactosylceramide β -galactosidase	Accumulation of galactosylceramides in liver and white matter, demyelination, failure of mental development.
Lactosylceramidosis	Ceramide lactosidase (β -galactosidase)	Accumulation of ceramide lactosides in liver, bone marrow and brain, cerebral degeneration, liver enlargement.
Metachromatic leucodystrophy	Aryl sulfatase	Accumulation of sulfatides in white matter, demyelination, failure of mental growth, psychopathic disorders.
Niemann-Pick disease	Sphingomyelinase	Accumulation of sphingomyelins in brain, liver and spleen, mental retardation, splenomegaly, liver enlargement, early death.
Tay-Sachs disease	β -N-Acetylhexosaminidase	Accumulation of GM ₂ gangliosides in brain and spleen, demyelination, swollen cerebral ganglion cells, mental retardation, severe motor deterioration, blindness, early death.

13.12 CHOLESTEROL METABOLISM

Synthesis

About 500 mg of cholesterol are normally synthesized in 24 hours from acetyl-CoA by an energy-consuming process of reductive synthesis, utilizing NADPH as the donor of reducing equivalents (H^+ and e^-). Each molecule of cholesterol is formed from 18 acetyl-CoA molecules. Feeding rats with acetate labelled with carbon isotopes, it has been shown that 12 of the carbons of cholesterol (C^2 , C^4 , C^6 , C^8 , C^{10} — C^{12} , C^{14} ,

C^{16} , C^{20} , C^{23} and C^{25}) come from the carboxyl carbons of acetate molecules while all the remaining 15 carbons of cholesterol come from the methyl carbons of acetate. Synthesis of each cholesterol molecule needs 13 NADPH molecules. To generate the latter, all steroidogenic tissues have a high rate of pentose phosphate pathway. Liver and intestinal mucosa synthesize respectively about 250 and 75 mg of cholesterol in 24 hours normally; the rest is formed largely in the skin and to a lesser extent, in the adrenal cortex and gonads. The process is extra-

mitochondrial and is carried out partly by cytosol enzymes and partly by membrane enzymes of smooth endoplasmic reticulum.

Reaction sequence :

1. *Mevalonate synthesis*: (a) In the cytosol, thiolase condenses two molecules of acetyl-CoA to acetoacetyl-CoA, releasing a coenzyme A molecule (Fig. 13.39). Alternatively, in extrahepatic tissues, acetoacetate is thioesterified to acetoacetyl-CoA by *acetoacetyl-CoA synthase* with the help of CoA and ATP. (b) *HMG-CoA synthase* of the cytosol condenses acetoacetyl-CoA with acetyl-CoA to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). (c) A microsomal sulfhydryl enzyme called *HMG-CoA reductase* uses NADPH as the electron-donor to reduce HMG-CoA in two steps to mevalonic acid. This is the *rate-limiting reaction* of cholesterol synthesis.

2. *Squalene synthesis*: (a) Mevalonate is converted to 3-phospho-5-pyrophosphomevalonate by three successive phosphorylations with ATP by the successive actions of three cytosolic enzymes, viz., *mevalonate kinase*, *phosphomevalonate kinase* and *5-pyrophosphomevalonate kinase* (Fig. 13.40). (b) Cytosolic *pyrophosphomevalonate decarboxylase*

cleaves 3-phospho-5-pyrophosphomevalonate into CO_2 , Pi and an isoprenoid called isopentenyl pyrophosphate (IPP). (c) Cytosolic *IPP isomerase* tautomerizes IPP into its isomer 3,3-dimethylallyl pyrophosphate (DPP). (d) Another cytosolic enzyme called *cis-prenyl transferase* condenses IPP (C_5) with DPP (C_5) to form geranyl pyrophosphate (C_{10}) and next condenses the latter with another IPP molecule to produce farnesyl pyrophosphate (C_{15}) made of 3 isoprenoid units. (e) An NADPH-dependent microsomal enzyme called *squalene synthase* condenses two farnesyl pyrophosphate molecules to form squalene (C_{30}) through an intermediate called presqualene pyrophosphate.

Farnesyl pyrophosphate has other alternative fates also. (i) Its farnesyl residue is incorporated into heme A of cytochrome oxidase to form an isoprenoid sidechain on the C^2 of heme A (Fig. 9.25). (ii) *cis-Prenyl transferase* condenses farnesyl pyrophosphate with upto 16 isopentenyl residues to form dolichol. (iii) *trans-Prenyl transferase* condenses farnesyl pyrophosphate with 3-7 isopentenyl residues to produce ubiquinones.

3. *Cholesterol formation*: This is carried out by microsomal enzymes in presence of

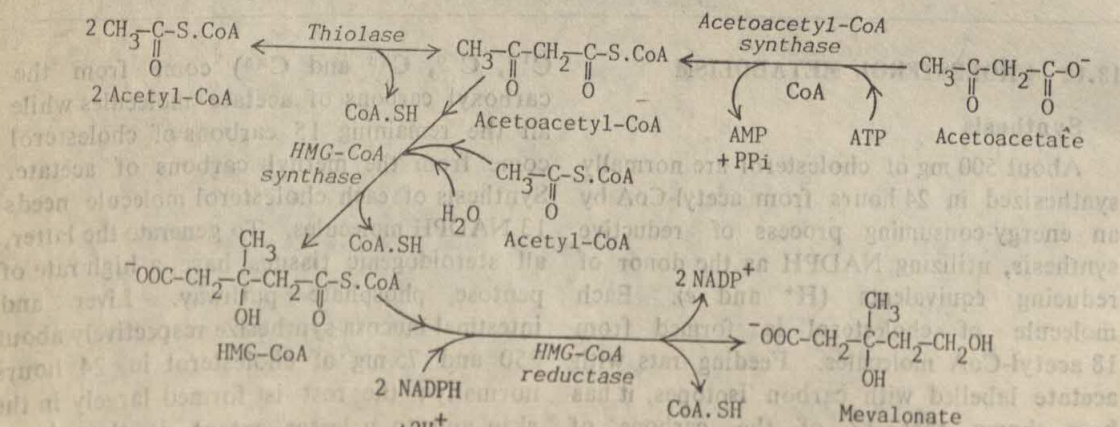


Fig. 13.39. Synthesis of mevalonate.

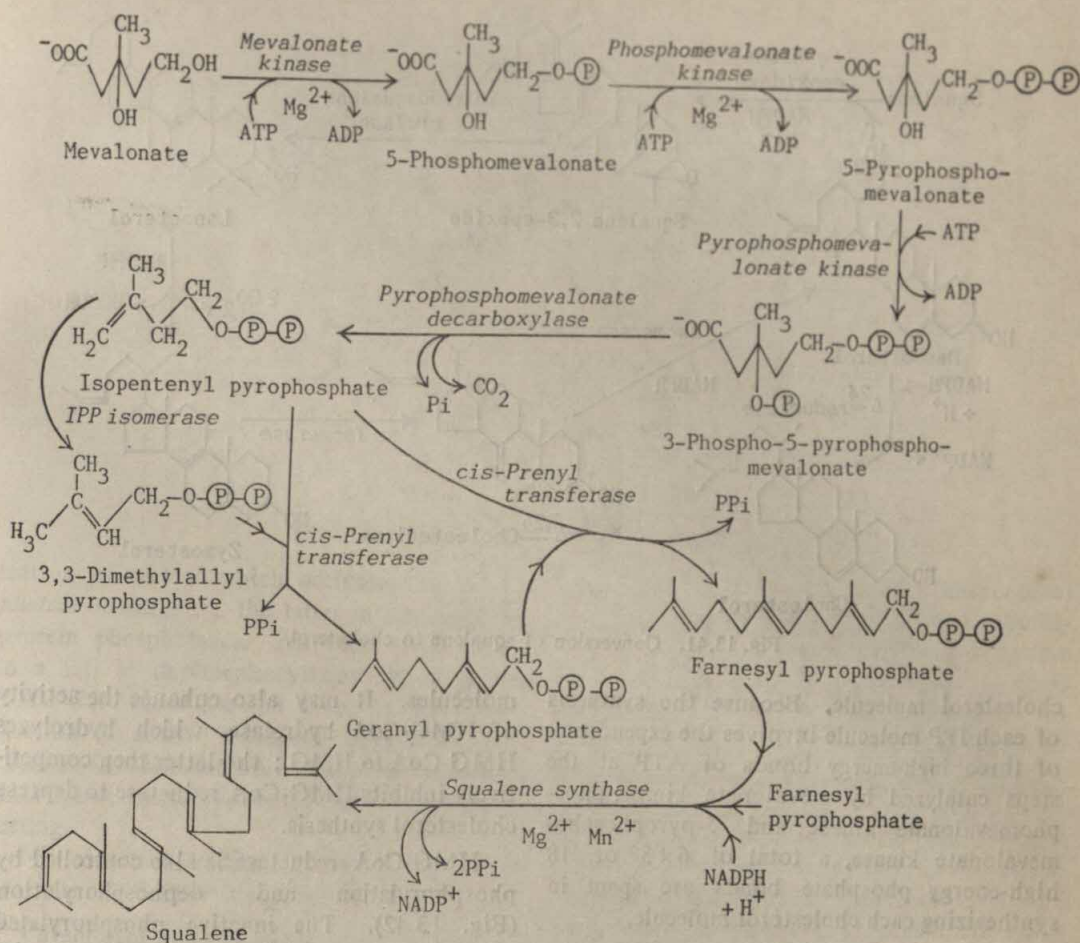


Fig. 13.40. Synthesis of squalene from mevalonate.

O₂. Squalene and other intermediates of this pathway remain bound to a *squalene-sterol carrier protein*. (a) *Squalene epoxidase* first oxidizes squalene to squalene 2,3-epoxide or 2,3-oxidosqualene with the help of molecular O₂ and NADPH. (b) *Oxidosqualene cyclase* cyclizes 2,3 oxidosqualene into lanosterol. (c) Microsomal enzymes oxidize the three methyl groups on C⁴ and C¹⁴ of lanosterol to liberate formate and two CO₂ and thereby convert lanosterol to zymosterol (Fig. 13.41). (d) A microsomal *isomerase* tautomerizes

zymosterol to $\Delta^{7,14}$ -cholestadienol. (e) The latter is further tautomerized to desmosterol with the help of O₂ and an NADPH-dependent enzyme. (f) A microsomal Δ^{24} -*reductase* uses NADPH as the electron-donor to reduce the Δ^{24} double-bond of desmosterol, producing cholesterol.

Energetics: Two farnesyl pyrophosphate molecules, each formed ultimately from three IPP molecules, are used in synthesizing one cholesterol molecule. So, a total of six IPP molecules are utilized in forming a

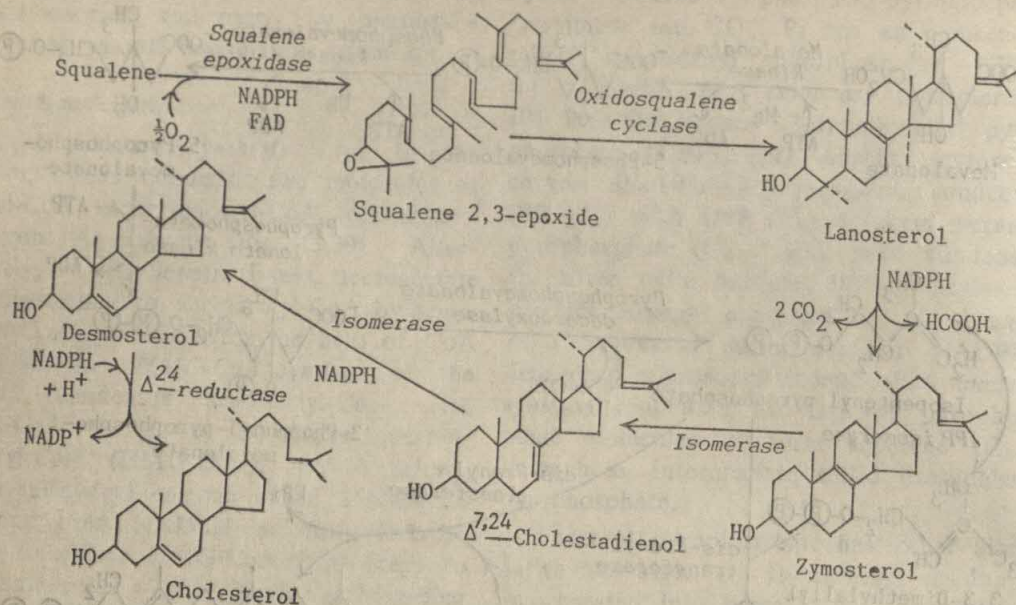


Fig. 13.41. Conversion of squalene to cholesterol.

cholesterol molecule. Because the synthesis of each IPP molecule involves the expenditure of three high-energy bonds of ATP at the steps catalyzed by mevalonate kinase, phosphomevalonate kinase and 5-pyrophosphomevalonate kinase, a total of 6×3 or 18 high-energy phosphate bonds are spent in synthesizing each cholesterol molecule.

Regulation ; The rate of cholesterol synthesis is controlled largely by regulating the activity of HMG-CoA reductase, its rate-limiting enzyme. High-cholesterol diet may cause a feedback inhibition of the hepatic synthesis of cholesterol while a low-cholesterol diet substantially enhances cholesterol synthesis in the liver, intestinal mucosa, skin and adrenal cortex. The inhibitory effect of cholesterol probably depends upon the repression of synthesis of HMG-CoA reductase by either cholesterol itself or oxysterol formed from it. Cholesterol may also induce the synthesis of enzymes which inactivate or degrade pre-existing reductase

molecules. It may also enhance the activity of HMG-CoA hydrolase which hydrolyzes HMG-CoA to HMG ; the latter then competitively inhibits HMG-CoA reductase to depress cholesterol synthesis.

HMG-CoA reductase is also controlled by phosphorylation and dephosphorylation (Fig. 13.42). The inactive phosphorylated form of reductase is dephosphorylated to its active form by *protein phosphatases* ; the active reductase is phosphorylated to the inactive reductase by *reductase kinase* and ATP. Insulin probably activates protein phosphatases ; these dephosphorylate inactive reductase to active reductase, and active reductase kinase to its inactive form. Both these effects increase the active reductase to enhance cholesterol synthesis.

Starvation reduces insulin secretion and enhances glucagon secretion. So, the activating action of insulin on protein phosphatases is reduced in starvation. On the other hand, glucagon increases the intracellular concen-

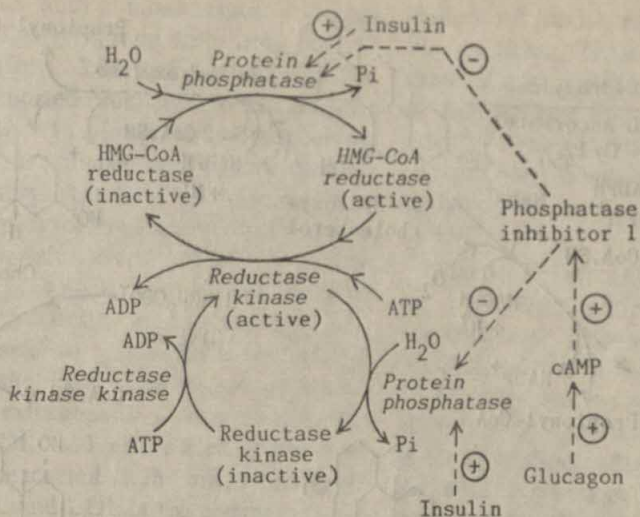


Fig. 13.42. Regulation of HMG-CoA reductase.

tration of cAMP which activates the *phosphatase inhibitor 1*; the latter in turn inhibits protein phosphatases. All these events lead to a fall in dephosphorylation of inactive HMG-CoA reductase and a rise in phosphorylation of active reductase, both causing a decline in HMG-CoA reductase activity. Cholesterol synthesis consequently falls during fasting.

Glucocorticoids like cortisol may also decrease HMG-CoA reductase activity.

Catabolism and excretion

Cholesterol is mainly excreted in the bile. It is carried in the nonaqueous central core of mixed micelles formed by bile salts and phosphatidylcholines in the bile. Most bile salts and some cholesterol of bile are reabsorbed from the intestine, the rest appearing in the feces. Putrefaction by colonic bacteria reduces some fecal cholesterol to coprostanol and cholestanol, and some bile salts to deoxycholate and lithocholate.

Synthesis of bile acids :

The main catabolic pathway of cholesterol proceeds through the synthesis of bile acids by microsomal enzymes of hepatocytes

(Fig. 13.43). (i) In the liver, microsomal 7α -hydroxylase hydroxylates the C 7 of cholesterol with the help of NADPH, cytochrome P_{450} , vitamin C and molecular O_2 to form 7α -hydroxycholesterol; atherosclerosis in scurvy results from the accumulation of cholesterol due to a failure of its catabolism to bile acids in absence of L-ascorbate. 7α -Hydroxylase is the *rate-limiting enzyme* of the process and is subject to feedback inhibition by bile acids. Decreased intestinal reabsorption of bile acids reduces their feedback inhibitory effect on 7α -hydroxylase and may thereby enhance bile acid synthesis. 7α -Hydroxylase is also controlled by phosphorylation and dephosphorylation—phosphorylated 7α -hydroxylase is far more active than its dephospho form. (ii) Other microsomal enzymes like the NADPH-dependent 12α -hydroxylase and a *thiokinase* help to change 7α -hydroxycholesterol to chylol-CoA and chenodeoxychylol-CoA through different pathways. (iii) *Cholyltransferases* then transfer either cholyl or chenodeoxychylol group from the respective CoA derivatives to glycine or taurine to produce primary bile acids such as glycocholic, taurocholic and

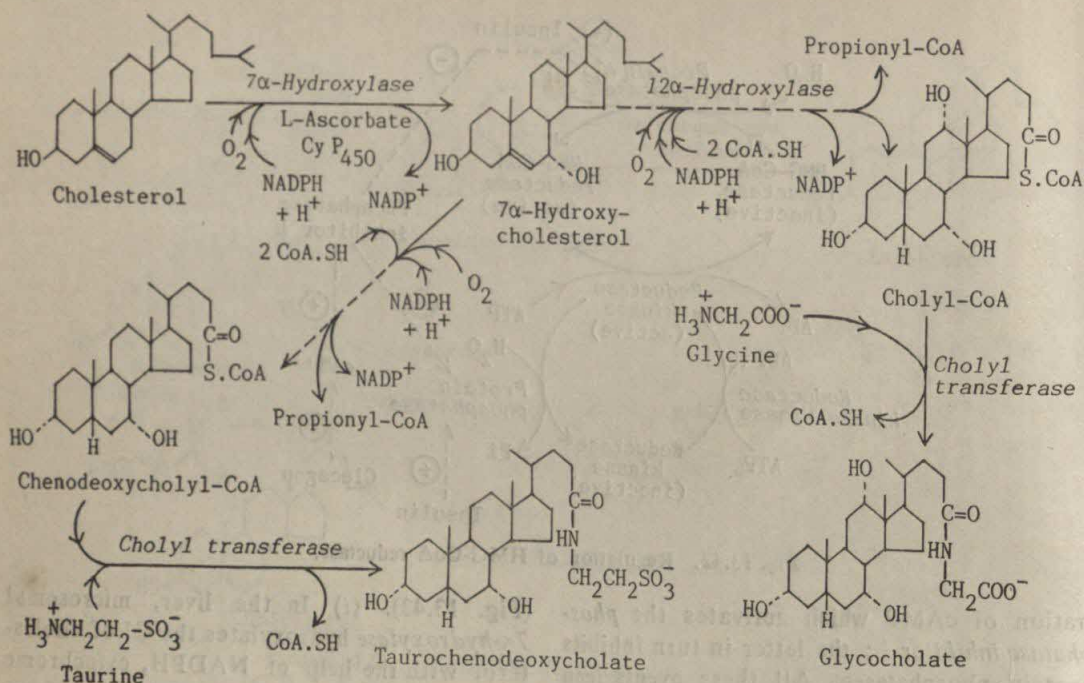


Fig.13.43. Synthesis of bile acids.

glycochenodeoxycholic acids. They occur in the bile largely as bile salts in association with sodium and potassium.

Transport

Each dl of human plasma normally contains a total of 150-265 mg of cholesterol; free and esterified cholesterol, both transported in plasma lipoproteins, constitute respectively

about $\frac{2}{3}$ rd- $\frac{3}{4}$ th and $\frac{1}{4}$ th- $\frac{1}{3}$ rd of total plasma cholesterol (Table 13.2).

Cholesterol is esterified in the plasma mainly by *lecithin-cholesterol acyltransferase* (LCAT) and in tissue cells mainly by *acyl-CoA cholesterol acyltransferase* (ACAT); these enzymes transfer respectively a polyunsaturated acyl (linoleyl) group from phos-

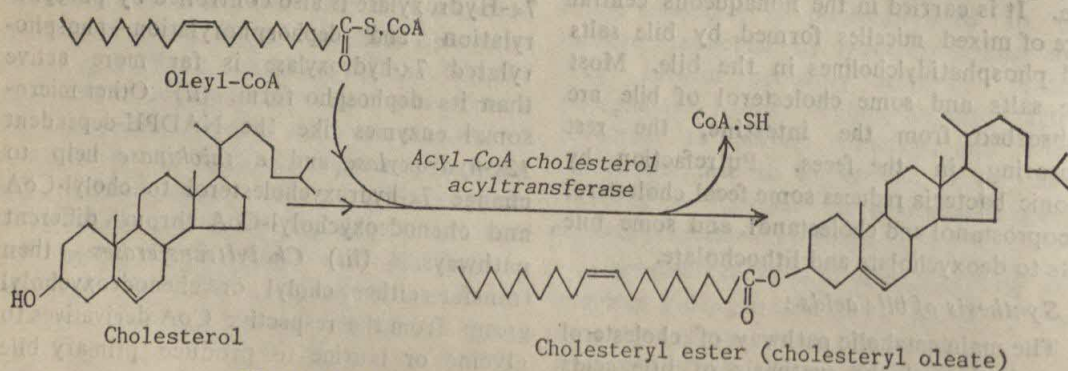


Fig. 13.44. Action of acyl-CoA cholesterol acyltransferase.

phatidylcholine and a monounsaturated acyl (oleyl or palmitoleyl) group from acyl-CoA to cholesterol (Figs. 13.33 and 13.44). Cholesterol is transported from liver to extrahepatic tissues mainly in low-density lipoproteins (LDL) which are pinocytosed by extrahepatic cells; cholesteryl linoleate of LDL is then hydrolyzed by lysosomal cholesterol esterase to give free cholesterol which is either incorporated in cellular membranes, or used in steroid synthesis, or re-esterified to cholesteryl oleate or palmitoleate by ACAT and stored in the cell. Cholesterol is transported from extrahepatic tissues to the liver mainly by HDL and either used in bile acid synthesis, or excreted in the bile, or recycled through VLDL and LDL in the plasma.

13.13 LIPOPROTEINS

Plasma lipoproteins

Many lipids such as triacylglycerols, higher saturated fatty acids and cholesteryl esters are insoluble in water. So, they are largely transported as water-miscible lipoproteins. Lipoprotein particles consist of varying amounts of lipids and proteins. The protein moieties, called *apolipoproteins*, and amphipathic lipids such as phospholipids and free

cholesterol constitute the surface layer of the particle. Their polar head-groups are directed on the water-adjoining outer surface of the particle and their nonpolar tails are oriented towards its nonaqueous central core occupied by nonpolar lipids such as triacylglycerols and cholesteryl esters (Fig. 13.45).

Plasma lipoproteins have been separated by *ultracentrifugation* into classes such as *chylomicrons*, *very low-density lipoproteins* (VLDL), *intermediate-density lipoproteins* (IDL), *low-density lipoproteins* (LDL) and *high-density lipoproteins* (HDL₂, HDL₃, etc.) in the ascending order of density. The higher the lipid percentage in a lipoprotein, the larger is its diameter, the lower its density and the higher its rate of floating, in Svedberg flotation units (S_f), through an NaCl solution of specific gravity 1.063 (Table 13.2). Lipoproteins have also been separated *electrophoretically* into classes such as α -*lipoproteins* (HDL), β -*lipoproteins* (LDL) and *pre- β -lipoproteins* (VLDL).

The principal apolipoprotein of chylomicrons is B-48 (MW 200 kdal); besides, they also carry A-I (28 kD), A-II (17 kD), C-II (8.8 kD), C-III (8.75 kD) and arginine-

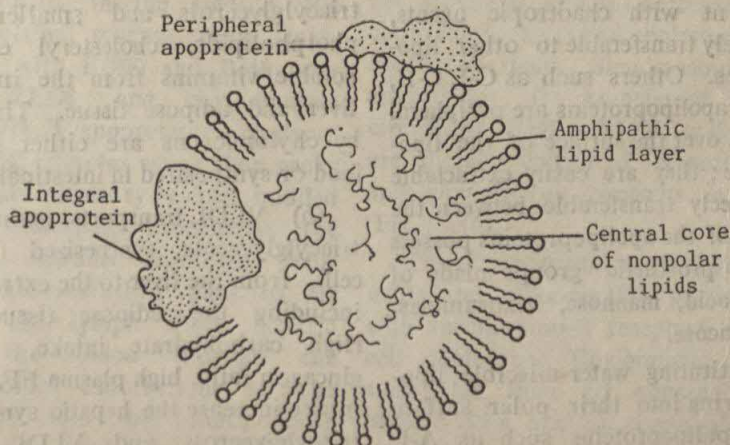


Fig. 13.45. Structure of a lipoprotein particle.

TABLE 13.2. Human plasma lipoproteins.

	Chylo- microns	VLDL	IDL	LDL	HDL ₂	HDL ₃	Albumin- FFA
Density (g cm ⁻³)	<0.94	0.94-1.006	1.006-1.019	1.019-1.063	1.063-1.125	1.125-1.210	>1.28
Flotation (S _f)	>400	20-400	12-20	2-12	<2		
Diameter (nm)	100-1000	30-80	25-30	20-25	10-20	7.5-10	
Amount (mg dl ⁻¹)	85-240	140-200	200-250	300-340	70-110	280-400	
Proteins (%)	2	8	10	22	33	56	99
Total lipids (%)	98	92	90	78	67	44	1
Triacylglycerols (%)	88	52	26	10	11	6	0
Phospholipids (%)	6	18	24	22	29	20	0
Total cholesterol (%)	4	21	39	45	27	15	0
Free cholesterol (%)	1	4	8	8	7	3	0
Cholesteryl esters (%)	3	17	31	37	20	12	0
Free fatty acids (%)	—	1	1	1	—	3	1

1 S_f = 10⁻¹¹ cm/s/dyne/g at 26°C.

rich E (34 kD) apoproteins. B-100 (350 kD) is the principal apolipoprotein of VLDL, IDL and LDL; besides, VLDL also contains C-I (7 kD), C-II, C-III and E apolipoproteins while IDL carries some E apoprotein. HDL carries A-I, A-II, C-I, C-II, C-III, D (32.5 kD) and E apoproteins. Some apolipoproteins (e.g., B apolipoproteins) are integral proteins embedded deep into the surface phospholipid layer of the droplet; they cannot be extracted from the lipoprotein droplets except by drastic treatment with chaotropic agents, nor are they freely transferable to other lipoprotein particles. Others such as C-I, C-II, C-III, D and E apolipoproteins are peripheral proteins, located over the surface of the lipoprotein particle; they are easily extractable from it and freely transferable between the particles. Some of the apolipoproteins possess oligosaccharide prosthetic groups made of L-fucose, sialic acid, mannose, hexosamines, galactose and glucose.

Besides constituting water-miscible lipoproteins by entering into their polar surface layer, some apolipoproteins such as A-I, C-I and C-II activate specific enzymes like

lipoprotein lipase and lecithin-cholesterol acyltransferase. Some like B-100 and E may bind with specific membrane receptors on hepatic cells, leading to the hepatic uptake of the corresponding lipoproteins. D apolipoprotein functions as the *cholesteryl ester transfer protein* for transferring cholesteryl esters between different lipoproteins.

Major functions

(a) Chylomicrons transport mainly triacylglycerols and smaller amounts of phospholipids, cholesteryl esters and fat-soluble vitamins from the intestine to the liver and adipose tissue. The lipids carried by chylomicrons are either obtained from food or synthesized in intestinal mucosa cells.

(b) VLDL transports mainly endogenous triacylglycerols, synthesized in the hepatic cells, from the liver to the extrahepatic tissues including the adipose tissue for storage. High carbohydrate intake, high insulin/glucagon ratio, high plasma FFA and alcohol intake increase the hepatic synthesis of both triacylglycerols and VLDL so that the enhanced amounts of fatty acids reaching the

liver may be speedily mobilized in VLDL to the adipose tissue.

(c) LDL, rich in cholesteryl esters, transports cholesterol and its esters from hepatic cells to extrahepatic tissues. LDL also regulates cholesterol synthesis in extrahepatic tissues, because the cholesterol delivered by LDL to the cells inhibits HMG-CoA reductase, the rate-limiting enzyme for cholesterol synthesis.

(d) HDL transports cholesterol and its esters from peripheral tissues to the liver. It also returns apolipoproteins to the liver.

(e) Albumin-FFA complexes transport mainly free fatty acids, released by adipose tissue lipolysis, and small amounts of lysophospholipids from extrahepatic tissues to the liver.

Metabolism

Chylomicrons and VLDL :

Chylomicrons and VLDL are synthesized in the intestinal mucosa and liver respectively. The polysomes on the rough endoplasmic reticulum (ER) of these tissues synthesize respectively B-48 and B-100 apolipoproteins. Triacylglycerols are synthesized in the smooth ER of both tissues while both microsomal and cytoplasmic enzymes participate in cholesterol synthesis. In the smooth ER of liver and intestinal mucosa, these lipids are incorporated with B-100 and B-48 apoproteins into VLDL and chylomicrons respectively. Their B apoproteins are glycosylated in the Golgi complex which then packs the lipoproteins into secretory vesicles budded off from Golgi cisternae. Eventually, the lipoproteins are extruded from the cells. Intestinal chylomicrons then pass to the plasma through the lymph while hepatic VLDL enters the blood through the fenestrated sinusoidal endothelium in the liver. After entry into the blood, these 'nascent' lipoproteins gradually acquire the full contingent of their apoproteins by the

transfer of C and E apoproteins from circulating HDL particles.

A *lipoprotein lipase* occurs on the membrane of capillary endothelial cells in extrahepatic tissues such as the adipose tissue, lactating mammary glands, heart, lungs, diaphragm and renal medulla. It hydrolyzes triacylglycerols of circulating VLDL and chylomicrons through di- and mono-acylglycerols to release free fatty acids and glycerol. Most of the released fatty acids are taken up by the tissue cells while smaller amounts may circulate in the plasma as albumin-FFA complexes. Lipoprotein lipase is induced by insulin and requires as cofactors phospholipids and C-II apolipoproteins, both present in its substrates VLDL and chylomicrons. Lipoprotein lipase activity declines in the adipocytes on starvation and rises after feeding; so, starvation reduces and feeding enhances the uptake and storage of fat by adipose tissue. On the contrary, starvation enhances lipoprotein lipase activity in cardiac and striated muscles, enabling them to take up and oxidize more fatty acids.

As lipoprotein lipase hydrolytically removes triacylglycerols from circulating VLDL and chylomicrons, the diameters and triacylglycerol contents of these lipoproteins get decreased and the percentage amounts of cholesteryl esters and cholesterol are almost doubled in them. Simultaneously, A and C apoproteins are transferred from them to circulating HDL. VLDL and chylomicrons are thereby converted respectively to IDL and *chylomicron remnants* in the plasma (Fig. 13.46).

The E apoproteins of chylomicron remnants and of some IDL particles may bind with specific apo-E receptors on the hepatic cell membrane. Chylomicron remnants and some IDL thus get concentrated on the hepatic cells and are eventually internalized into them by pinocytosis. Hepatic cells hydrolyze triacylglycerols and cholesteryl esters

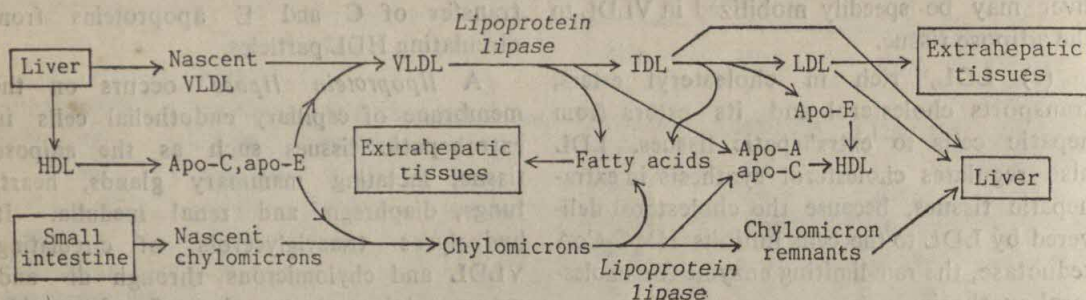


Fig. 13.46. Metabolic fates of chylomicrons and VLDL.

of those lipoproteins into fatty acids, cholesterol and glycerol for further metabolism.

Low-density lipoproteins:

Most IDL particles change into LDL by losing their E apoproteins as also some triacylglycerols. This makes LDL richer in cholesteryl esters and cholesterol, poorer in triacylglycerols and total lipids, smaller in diameter and higher in density than IDL. Cholesteryl linoleate, containing the polyunsaturated linoleic acid, is the principal cholesteryl ester in LDL. The B-100 apoprotein of circulating LDL binds with specific B-100 receptors (*LDL receptors*) located on the plasma membrane of depressed *coated-pit regions* (Fig. 13.47) on the surface of hepatic cells, lymphoid cells, fibroblasts and smooth muscle fibres. Thus, LDL particles get concentrated in the coated-pit regions which

are invaginated into the cytoplasm and ultimately pinched off to form coated vesicles first and then endosome vesicles rich in LDL (*adsorptive pinocytosis*). The endosome vesicle ultimately fuses with a lysosome. Lysosomal acid proteases hydrolyze the B apoprotein of LDL into amino acids while a lysosomal acid cholesterol esterase hydrolyzes cholesteryl esters of LDL into cholesterol and fatty acids.

In this way, most of the circulating LDL delivers cholesterol to extrahepatic tissues. They either store the cholesterol as cholesteryl oleate and palmitoleate after its esterification with monounsaturated oleic and palmitoleic acids by *acyl-CoA cholesterol acyltransferase*, or incorporate the cholesterol into the lipid bilayer of cellular membranes. A rise in intracellular cholesterol inhibits the synthesis of new LDL receptors, consequently decreases their density on the plasma membrane and thereby lowers the cellular intake of LDL-cholesterol from the plasma. A rise in intracellular cholesterol inhibits HMG-CoA reductase also and thereby decreases cholesterol biosynthesis in the cell. Thus, LDL regulates the rate of cholesterol biosynthesis in extrahepatic tissues by delivering hepatic cholesterol to those tissues.

The rest of the circulating LDL, pinocytized by hepatic cells, delivers its cholesterol to the liver for either catabolism to bile acids or excretion in the bile.

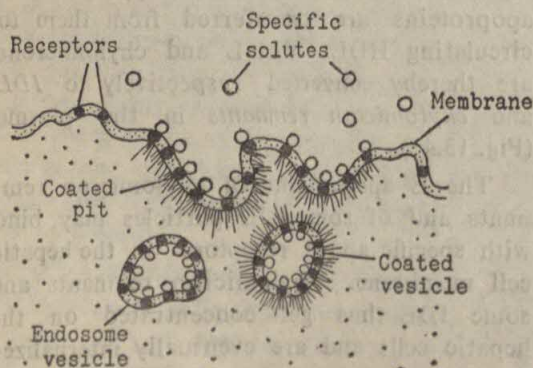


Fig. 13.47. Adsorptive pinocytosis.

High-density lipoproteins :

In the liver, A and C apolipoproteins are synthesized by polysomes on the rough ER and are assembled with lipids into nascent HDL particles in the smooth ER. Thus, the liver releases 'nascent' HDL, containing both A and C apoproteins, into the plasma (Fig. 13.48). But nascent HDL, synthesized and released by small intestine, contains only A apoproteins; only when circulating in the plasma, intestinal HDL acquires C apolipoproteins synthesized in the liver. Nascent HDL is made of a bilayer of phospholipids and cholesterol, arranged in a disc-like form.

It may be recalled that HDL transports cholesterol and cholesteryl esters from extrahepatic tissues to the liver. (a) *Lecithin-cholesterol acyltransferase* (LCAT) of plasma binds with the nascent HDL disc, gets activated by A-I and C-I apoproteins of HDL itself, and transfers acyl groups from HDL phospholipids to free cholesterol to produce lysophospholipids and cholesteryl esters (Fig. 13.33). Lysophospholipids are released from HDL to plasma where they bind to albumin; cholesteryl esters are transferred into the central core of the HDL particle. This gradually changes the discoid nascent HDL into a spherical HDL₃ particle

bounded by a lipid layer with apoproteins embedded in it. (b) Cholesterol, released from chylomicrons and VLDL during extrahepatic *lipoprotein lipase* action, is also accepted by circulating HDL with the help of HDL-bound LCAT. The latter esterifies cholesterol into cholesteryl esters in HDL and thus maintains a low concentration of free cholesterol in HDL, enabling the transfer of more cholesterol into the latter. HDL₃ is thus changed ultimately to HDL₂, richer in cholesteryl esters. (c) D apoprotein of HDL functions as the *cholesteryl ester transfer protein* and transfers some cholesteryl esters from HDL to VLDL, LDL and chylomicrons in the plasma; these lipoproteins may then transport those cholesteryl esters to liver. Some C and E apoproteins are also transferred from circulating HDL to VLDL and chylomicrons. (d) *Heparin-releasable hepatic lipase*, released from the liver by large quantities of heparin, hydrolyzes surface phospholipids of HDL₂ particles reaching the liver, and thereby enables the hepatic uptake of cholesterol, its esters and apolipoproteins from HDL₂.

Clinical conditions

It may be recalled that cholesterol and triacylglycerols released by the liver are

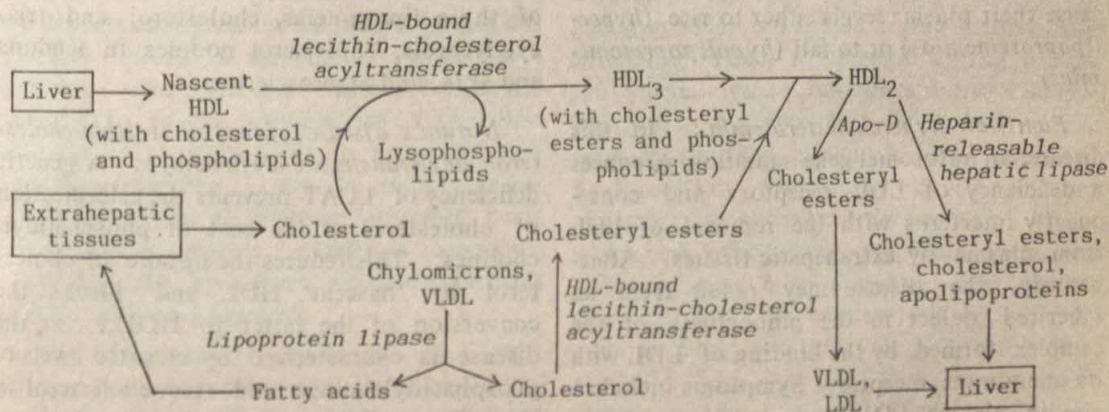


Fig. 13.48. Metabolic fates of HDL.

transported to extrahepatic tissues mainly in LDL and VLDL respectively; on the contrary, HDL₂ mainly helps to remove cholesterol from blood and extrahepatic tissues by carrying it to the liver. So, a prolonged rise in plasma LDL, VLDL or LDL-cholesterol/HDL-cholesterol ratio, produces protracted high plasma levels of cholesterol and triacylglycerols. This often leads to the deposition of cholesteryl ester plaques on arterial walls, producing atherosclerosis and coronary occlusion diseases. Estimation of plasma LDL-cholesterol is, therefore, of considerable importance in cardiovascular disorders. Prolonged rise in plasma LDL-cholesterol and VLDL may occur in diabetes mellitus, hypothyroidism, excessive coffee and nicotine intake or habitual intake of foods rich in saturated fatty acids. VLDL particles, when formed with saturated fatty acids, are richer in cholesteryl esters of saturated fatty acids which are only slowly removed from the blood. In contrast, polyunsaturated fatty acid esters of cholesterol are more rapidly removed from the blood and metabolized; so, polyunsaturated fatty acid intake may lower the plasma LDL-cholesterol.

Dyslipoproteinemias :

Following inborn errors in the synthesis, transport or removal of lipoproteins may cause their plasma levels either to rise (*hyperlipoproteinemias*) or to fall (*hypolipoproteinemias*).

Familial hypercholesterolemia : In this disease, an autosomal gene mutation produces a deficiency of LDL receptors and consequently interferes with the removal of LDL from plasma by extrahepatic tissues. Alternatively, the disease may result from an inherited defect in the pinocytosis of the complex formed by the binding of LDL with its membrane receptor. Symptoms include a rise in plasma LDL (*hypobetalipoproteinemia*), high serum cholesterol, cholesterol

nodules in tendons and skin, cholesterol plaques on arterial walls and atherosclerosis.

Cholesteryl ester storage disease (Wolman's disease) : A genetic deficiency of lysosomal cholesterol esterase in extrahepatic cells like fibroblasts produces a failure to metabolize cholesteryl esters of LDL. This leads to a high blood cholesterol and atherosclerosis.

Familial lipoprotein lipase deficiencies : An inborn deficiency of either lipoprotein lipase or the C-II apolipoprotein acting as its cofactor, may decrease the rate of extrahepatic removal of VLDL and chylomicrons from the plasma through the hydrolysis of their triacylglycerols. This increases the plasma levels of those two lipoproteins.

Abetalipoproteinemia and hypobetalipoproteinemia : see page 220.

Familial hypertriacylglycerolemia : In this disease, VLDL, triacylglycerol and cholesterol levels rise in blood, but LDL and HDL levels fall.

Tangier disease (familial alpha-lipoprotein deficiency) : see page 220.

Familial dysbetalipoproteinemia : A genetic defect in E apolipoprotein blocks the normal metabolism of chylomicron remnants and IDL. This causes elevated plasma levels of those lipoproteins, cholesterol and triacylglycerols, cholesterol nodules in tendons and skin, and atherosclerosis.

Norum's disease (familial lecithin-cholesterol acyltransferase deficiency) : A genetic deficiency of LCAT prevents the esterification of cholesterol at the cost of phosphatidylcholines. This reduces the uptake of cholesterol by 'nascent' HDL and blocks the conversion of the latter to HDL₂. So, the disease is characterized by elevated levels of phosphatidylcholines and free cholesterol in the plasma, fall in plasma cholesteryl esters, lysolecithins and α -lipoproteins (HDL₂) and

appearance of abnormal aggregates of discoidal 'nascent' HDL in the plasma.

13.14 DEPOT FAT

Fat is stored in the mammalian adipose tissue mainly as *white fat*. Some *brown fat* may occur around visceral organs. In contrast to white fat, adipocytes of brown adipose tissue possess more numerous mitochondria, more abundant mitochondrial cytochromes and higher O_2 consumption rates, but poorer ATP synthase activity. Brown fat primarily helps in heat production during cold exposure and arousal from hibernation.

There are two different pools of fatty acids in adipocytes. Fatty acids of one pool are collected by adipocytes from plasma following their release from triacylglycerols of circulating VLDL and chylomicrons by the action of lipoprotein lipase. Fatty acids of the other pool are continually derived from the hydrolysis of triglycerides stored in adipocytes. Fatty acids of this pool are released in the plasma which transports them to liver and other tissues as albumin-fatty acid complexes. Besides, fatty acids of both pools may be either oxidized or re-esterified to triacylglycerols and stored in adipocytes.

Adipose tissue lipolysis

Lipolysis (hydrolysis) of stored triacylglycerols is catalyzed by the successive actions of three lipases in adipocytes. A *hormone-sensitive triacylglycerol lipase*, generally referred to as the adipose tissue lipase, first hydrolyzes triacylglycerols to fatty acids and diacylglycerols (Fig. 13.49). A *diacylglycerol lipase* hydrolyzes diacylglycerols to fatty acids and monoacylglycerols. Finally, a *monoacylglycerol lipase* hydrolyzes monoacylglycerols to fatty acids and glycerol.

The triacylglycerol lipase has far lower activity than the other two lipases. It is a regulated enzyme and the reaction, catalyzed by it, is the *rate-limiting step* of adipose tissue

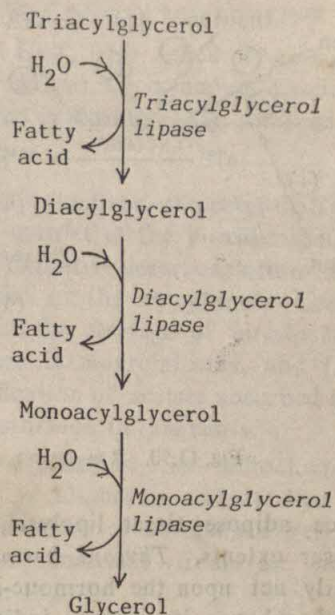


Fig. 13.49. Adipose tissue lipolysis.

lipolysis. It exists in an active phosphorylated *a* form and an inactive dephosphorylated *b* form and is largely regulated by phosphorylation and dephosphorylation. A cAMP-dependent *protein kinase* uses ATP to phosphorylate inactive lipase *b* to active lipase *a*; a *lipase phosphatase* dephosphorylates active lipase *a* to inactive lipase *b* (Fig. 13.50).

Major *lipolytic hormones* consist of *glucagon* which is secreted during fasting, and *adrenaline* which is secreted during exercise and stress. These hormones activate *adenylate cyclase* in adipocytes. Adenylate cyclase increases the intracellular cAMP by catalyzing its formation from ATP. cAMP in turn allosterically activates the protein kinase which phosphorylates the inactive triacylglycerol lipase *b* to form the active lipase *a* (Fig. 13.50). This enhances lipolysis in the adipose tissue and raises the plasma free fatty acid (FFA) level during fasting, exercise and stress. *ACTH*, *TSH*, β -*MSH* and *vasopressin* may also activate the lipase in a similar way

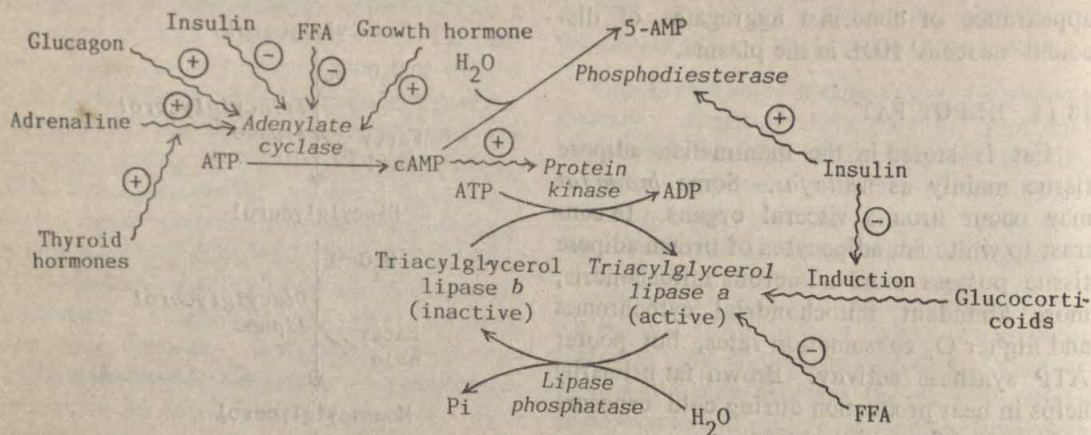


Fig. 13.50. Regulation of the hormone-sensitive triacylglycerol lipase.

to enhance adipose tissue lipolysis, but to much lesser extents. *Thyroid hormones* do not directly act upon the hormone-sensitive lipase, but enhance its activity indirectly by facilitatory effects on the actions of other lipolytic hormones. *Growth hormone*, on the other hand, stimulates the synthesis of proteins participating in cAMP formation, thereby increasing the intracellular cAMP in adipocytes and consequently enhancing lipolysis. *Glucocorticoids*, secreted in stress and starvation, enhance adipose tissue lipolysis by inducing the synthesis of the hormone-sensitive lipase.

Insulin lowers the cAMP concentration in adipocytes by inhibiting adenylate cyclase which forms cAMP, and also by activating a phosphodiesterase which degrades cAMP to 5'-AMP. Insulin also inhibits the glucocorticoid-induced synthesis of the hormone-sensitive lipase. All these decrease the activity of that lipase to depress lipolysis and thereby lower the plasma FFA level. Activation of the lipase increases abnormally in diabetes, raising the plasma FFA level.

The hormone-sensitive lipase is also inhibited by the feedback effect of high FFA concentrations. High levels of FFA may

also reduce the lipase activity by lowering the intracellular cAMP through an inhibitory action on adenylate cyclase.

13.15 FATTY LIVER

Triacylglycerols and fatty acids, synthesized or taken up by the liver, are rapidly incorporated into lipoproteins and then mobilized to extrahepatic tissues through the plasma mainly as VLDL. However, under the following conditions, the liver gets loaded with too much lipids (*fatty liver*), leading to the fibrosis of hepatocytes, cirrhosis and hepatic failure.

(a) Plasma levels of free fatty acids may rise due to either a high-fat diet or an increased lipolysis of adipose tissue fat during starvation and diabetes. The liver then takes up far more fatty acids and produces far more triacylglycerols from them than what it can incorporate into lipoproteins like VLDL for mobilization to extrahepatic tissues. This may accumulate too much fat in the liver.

(b) Fatty liver may result from a failure to synthesize VLDL in consequence of a dietary deficiency of choline, essential fatty acids or methionine, because choline and essential fatty acids are essential constituents

of phospholipids used in VLDL formation while methionine is the methyl-donor for choline synthesis. A high-cholesterol diet may produce fatty liver because cholesterol binds up essential fatty acids in cholesteryl esters and reduces their availability for VLDL synthesis. Choline, methionine, betaine and essential fatty acids can prevent fatty liver by promoting fat mobilization from the liver in the form of VLDL and are thus called *lipotropic factors*.

(c) Administration of puromycin or poisoning with CCl_4 , CHCl_3 , lead, phosphorus or arsenic may cause fatty liver by blocking the synthesis of apolipoprotein B-100 in the liver and consequently reducing VLDL synthesis.

(d) Orotate produces fatty liver by inhibiting the glycosylation of B-100 apolipoprotein of VLDL in the Golgi cisternae and consequently preventing the hepatic secretion of VLDL into the plasma.

(e) CCl_4 may give rise to free radicals which peroxidate the membrane lipids of hepatic smooth endoplasmic reticulum. This blocks the conjugation of lipids with apolipoproteins for the formation of VLDL and also prevents VLDL secretion. Both may lead to fatty liver.

13.16 TWO-CARBON METABOLISM

Acetyl-CoA, also called "active acetate", is the principal C_2 compound produced and metabolized in the body (Fig. 13.51).

Origin

The major sources of acetyl-CoA in mitochondria consist of the β -oxidation of fatty acids and oxidative decarboxylation of pyruvate. Most of the cytoplasmic acetyl-CoA comes from the cleavage of citrate by ATP-citrate lyase in nonruminants, and from the thioesterification of acetate absorbed from the ruminant stomach in ruminants.

(a) β -oxidation: In mitochondria of hepatocytes, adipocytes and cardiac muscle cells, each cycle of β -oxidation releases an acetyl-CoA molecule from an acyl-CoA (Fig. 13.5).

(b) *Oxidative decarboxylation of pyruvate*: Pyruvate dehydrogenase oxidatively decarboxylates pyruvate into acetyl-CoA in particularly hepatic and cardiac mitochondria (Fig. 7.9).

(c) *Cleavage of citrate*: ATP-citrate lyase cleaves citrate into oxaloacetate and acetyl-CoA in the cytoplasm of liver, adipose tissue, intestinal mucosa and kidneys with the help of ATP and coenzyme A (Fig. 13.12).

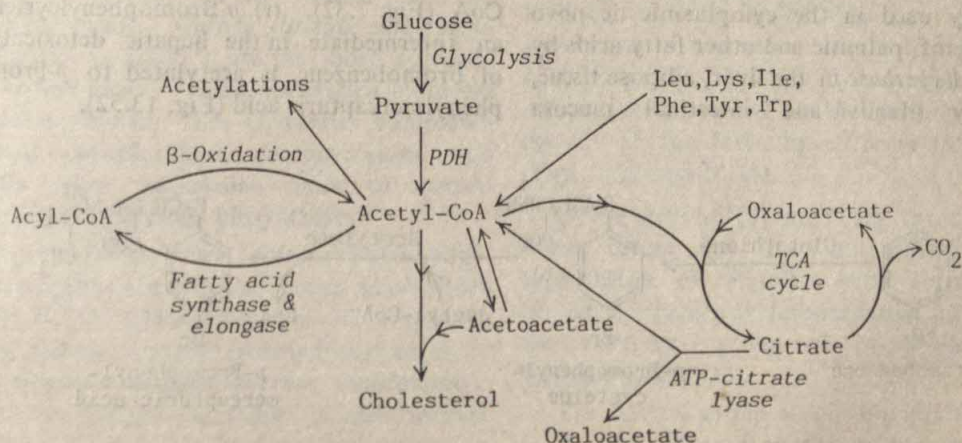


Fig. 13.51. Summary of acetate metabolism.

(d) *Thioesterification of acetate*: Acetate, derived from cellulose fermentation in ruminants, is thioesterified to acetyl-CoA by cytoplasmic *acetyl-CoA synthase* (Fig. 13.13). Small amounts of acetate, derived from the catabolism of threonine, oxidation of ethanol or hydrolysis of aspirin, may be similarly changed to acetyl-CoA.

(e) *Ketone body metabolism*: In extrahepatic tissues like muscles, *acetoacetyl-CoA synthase* uses CoA to thioesterify acetoacetate to acetoacetyl-CoA which is then cleaved into two acetyl-CoA molecules by CoA and *thiolase* (Fig. 13.8).

(f) *Amino acid catabolism*: Ketogenic and glycolytic-ketogenic amino acids, viz., Leu, Lys, Ile, Phe, Tyr and Trp, produce acetyl-CoA either directly or through acetoacetate (Figs. 12.39, 12.44-12.47).

Fate and functions

(a) *TCA cycle*: Most of the acetyl-CoA is ordinarily oxidized in the TCA cycle through the formation of citrate (pages 329-331). Oxidation of each acetate molecule in this cycle produces 12 high-energy bonds of ATP. This aerobic oxidation of acetate is the major source of energy in aerobic tissues.

(b) *Fatty acid synthesis*: (i) Acetyl-CoA is largely used in the cytoplasmic *de novo* synthesis of palmitic and other fatty acids by *fatty acid synthase* in the liver, adipose tissue, mammary glands and intestinal mucosa

(pages 411-416). (ii) *Microsomal elongase system* uses malonyl-CoA, synthesized from acetyl-CoA, to elongate pre-existing acyl-CoA molecules by C_2 units (pages 418-419). (iii) *Mitochondrial elongase system* uses acetyl-CoA itself in incorporating C_2 units into acyl-CoA (pages 419-420).

(c) *Carboxylation*: Acetyl-CoA is carboxylated to malonyl-CoA by *acetyl-CoA carboxylase* with the help of ATP, HCO_3^- and biotin (Fig. 13.15). Malonyl-CoA is used by fatty acid synthase and by microsomal elongase system in synthesizing fatty acids.

(d) *Acetylations*: Acetyl transferases (acetylases) transfer the acetyl group from acetyl-CoA to many substrates. Examples: (i) In the cholinergic neuron, *choline acetylase* transfers the acetyl group of acetyl-CoA to choline to form acetylcholine (Fig. 7.32). (ii) In the pineal gland, *serotonin N-acetylase* transfers the acetyl group from acetyl-CoA to serotonin to form N-acetylserotonin for melatonin synthesis (Fig. 12.21). (iii) In the skin, liver, bones and cartilages, *glucosamine 6-phosphate acetylase* uses the acetyl group of acetyl-CoA to acylate glucosamine 6-phosphate to N-acetylglucosamine 6-phosphate (Fig. 11.42). (iv) Sulfanilamide is detoxicated to N-acetylsulfanilamide in the liver by *sulfanilamide acetylase* and acetyl-CoA (Fig. 7.32). (v) *p*-Bromophenylcysteine, an intermediate in the hepatic detoxication of bromobenzene, is acetylated to *p*-bromophenylmercapturic acid (Fig. 13.52).

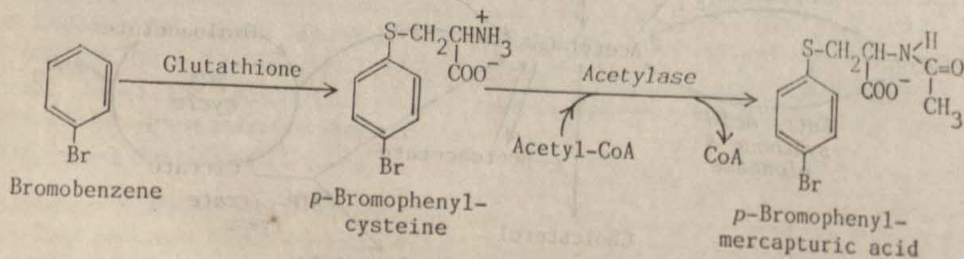


Fig. 13.52. Acetylation of *p*-bromophenylcysteine to *p*-bromophenylmercapturic acid.

(e) *Ketogenesis*: In starvation, carbohydrate deprivation, diabetes or any disturbance of the TCA cycle, acetyl-CoA from β -oxidation fails to be oxidized, accumulates in the liver and gets condensed in pairs by β -ketothiolase to produce acetoacetate (Fig. 13.6). In extrahepatic tissues, acetoacetate is reconverted to acetyl-CoA which is oxidized for energy.

(f) *Cholesterol synthesis*: In the cytoplasm of liver, intestinal mucosa, skin, adrenal cortex and gonads, cytosolic and microsomal enzymes use acetyl-CoA and acetoacetyl-CoA, formed from the latter, to synthesize cholesterol (see pages 439-441). Each cholesterol molecule is synthesized from 18 acetyl-CoA molecules.

13.17 ONE-CARBON METABOLISM

One-carbon units exist at different oxidation levels in the animal body. The methyl group ($-\text{CH}_3$), the most reduced one-carbon unit, is carried by tetrahydrofolate, methionine, betaine, choline and cobalamin; e.g., N^5 -methyl H_4 folate, S-adenosylmethionine and methylcobalamin. The methylene group ($-\text{CH}_2-$), a less reduced form, is carried as $\text{N}^5, \text{N}^{10}$ -methylene H_4 folate. The methenyl ($-\text{CH} =$), formyl ($-\text{CHO}$) and formimino ($-\text{CH} = \text{NH}$) groups are more oxidized forms. They are carried on N^5 and/or N^{10} of tetrahydrofolate. Carbon dioxide and carboxylate ion ($-\text{COO}^-$) are the most oxidized one-carbon units. These are carried by biotin as carboxybiotin. The C_1 -carrier complexes undergo considerable interconversions and donate their one-carbon units to specific substrates for various biosyntheses.

$\text{N}^5, \text{N}^{10}$ -Methylene H_4 folate: Tetrahydrofolate acquires a methylene group from either (a) the β -C of serine or (b) the α -C of glycine by the actions of *serine transhydroxymethylase* and *glycine cleavage enzyme* respectively; $\text{N}^5, \text{N}^{10}$ -methylene H_4 folate is also formed by either (c) the NAD^+ -dependent oxidation

of N^5 -methyl H_4 folate or (d) the NADPH-mediated reduction of $\text{N}^5, \text{N}^{10}$ -methenyl H_4 folate (Figs. 7.25, 12.29-12.30).

$\text{N}^5, \text{N}^{10}$ -Methylene H_4 folate serves as a C_1 -donor in (i) the methylation of deoxyuridylylate (dUMP) to deoxythymidylylate (dTTP) by *thymidylylate synthase*, (ii) the synthesis of glycine by *glycine synthase* and (iii) the synthesis of serine from glycine by *serine transhydroxymethylase* (Figs. 12.29, 12.30 and 14.6). Through these reactions, the methylene-C of $\text{N}^5, \text{N}^{10}$ -methylene H_4 folate serves as the source of the methyl-C of thymine, the α -C of glycine and the β -C of serine, respectively. The methylene group of $\text{N}^5, \text{N}^{10}$ -methylene H_4 folate is also (iv) oxidized to form the methenyl group of $\text{N}^5, \text{N}^{10}$ -methenyl H_4 folate or (iv) reduced to the methyl group of N^5 -methyl H_4 folate (Fig. 7.25).

$\text{N}^5, \text{N}^{10}$ -Methenyl H_4 folate: This is formed either (a) from $\text{N}^5, \text{N}^{10}$ -methylene H_4 folate by the NADP^+ -mediated oxidation of the methylene group, or (b) from N^5 -formimino H_4 folate by the deamination of its formimino group, or (c) from N^5 -formyl H_4 folate by an ATP-dependent *cyclohydrazase* (Fig. 7.25).

Its methenyl group may be (i) reduced to the methylene group of $\text{N}^5, \text{N}^{10}$ -methylene H_4 folate by an NADPH-dependent dehydrogenase, or (ii) changed to the formyl group of N^{10} -formyl H_4 folate by a *cyclohydrolase*, or (iii) incorporated in the purine ring as the C^8 of the latter by a *formyltransferase* (Figs. 7.25 and 14.11).

N^{10} -Formyl H_4 folate: Its formyl group arises either (a) from an ATP-dependent formylation of H_4 folate with formate, or (b) by a *cyclohydrolase*-mediated change in the methenyl group of $\text{N}^5, \text{N}^{10}$ -methenyl H_4 folate (Fig. 7.25).

The formyl group of N^{10} -formyl H_4 folate is (i) incorporated in the purine ring as its

C², or (ii) shifted to N⁵ of the folate by an isomerase to produce N⁵-formyl H₄ folate.

N⁵-Formyl H₄ folate or folinic acid: The formyl group of this compound is transferred by *formylglutamate formyltransferase* to the amino-N of glutamate to produce N-formylglutamate and H₄ folate.

N⁵-Formimino H₄ folate: The formimino group of N-formiminoglutamate (figlu), formed from histidine, is transferred by *figlu formiminotransferase* to H₄ folate to produce glutamate and N⁵-formimino H₄ folate (Fig. 12.41). The formimino group of the latter is deaminated into a methenyl group, producing N⁵,N¹⁰-methenyl H₄ folate (Fig. 7.25).

N⁵-Methyl H₄ folate and methylcobalamin: N⁵-Methyl H₄ folate is produced by the NADPH-mediated reduction of the methylene group of N⁵,N¹⁰-methylene H₄ folate (Fig. 7.25). Its methyl group serves as the ultimate source of the methyl group of methionine. *Homocysteine methyltransferase* transfers the methyl group first from N⁵-methyl H₄ folate to cobalamin, producing methylcobalamin which then donates its methyl group to homocysteine to change the latter to methionine (Fig. 7.26).

S-Adenosylmethionine: Compounds such as methionine, N⁵-methyl H₄ folate, betaine and cobalamin carry methyl groups which can be transferred by methyltransferases to other substances (*labile methyl groups*). Of such methyl donors, methionine is of prime importance. Methionine is formed in the cytoplasm by the methylation of homocysteine with either (a) the methyl group of N⁵-methyl H₄ folate through methylcobalamin acting as an intermediate carrier, or (b) a methyl group from betaine (Fig. 7.26). Methionine then changes to S-adenosylmethionine by receiving an adenosyl group from ATP under the action of *methionine adenosyl transferase* (Fig. 12.32). S-Adenosylmethionine has a higher transfer potential and serves as a

methyl donor to various substrates under the action of specific *methyltransferases*. The methyl group of S-adenosylmethionine thus finds its way into the molecules of adrenaline, melatonin, creatine phosphate, betaine, choline, phosphatidylcholine, N-methylnicotinamide, N-methylhistamine, etc. (pages 385-386).

Carboxybiotin: ATP-dependent carboxylases such as *pyruvate carboxylase*, *acetyl-CoA carboxylase* and *propionyl-CoA carboxylase* first use HCO₃⁻ and ATP to form a carbonic-phosphoric anhydride. The carboxylate ion (COO⁻) is next transferred from the latter to the N¹ of the biotin prosthetic group of the carboxylase, producing carboxybiotin ('active' CO₂). Each carboxylase then transfers the carboxylate ion from carboxybiotin to its specific substrate, carboxylating the latter (page 155). In this way, the one-carbon carboxylate ion finds its way into the carboxyl groups of oxaloacetate, malonyl-CoA and methylmalonyl-CoA respectively.

Carbamoyl phosphate: This is an important one-carbon compound, formed in the body from HCO₃⁻. (a) The cytoplasmic *carbamoyl phosphate synthase II* utilizes HCO₃⁻, a phosphate from ATP and the amide-N from glutamine to produce carbamoyl phosphate. The carbamoyl group of the latter is transferred by *aspartate transcarbamoylase* to aspartate (Fig. 12.16); carbamoylaspartate thus produced is used in pyrimidine synthesis. In this way, the carbamoyl group, formed in the cytoplasm, serves as the source of C² of the pyrimidine ring (Fig. 14.2). (b) The mitochondrial *carbamoyl phosphate synthase I* uses HCO₃⁻, free NH₄⁺ and a phosphate from ATP to produce carbamoyl phosphate. The carbamoyl group of the latter is transferred by *ornithine transcarbamoylase* to ornithine (Fig. 12.12); citrulline produced thereby is used in urea synthesis. Thus, the one-carbon carbamoyl group, formed in mitochondria, serves as the source of the urea-C.

It may also be recalled that the *bicarbonate buffer* of the body is a mixture of two one-carbon compounds, viz., HCO_3^- and H_2CO_3 . They are formed by a reaction between H_2O and CO_2 , catalyzed by *carbonic anhydrase* in erythrocytes, parietal cells and renal tubule cells: $\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$.

13.18 SUMMARY OF INTERMEDIARY METABOLISM

Intermediary metabolism consists of the metabolic fates of food components after their absorption into the body. Carbohydrates, lipids and proteins are absorbed in nonruminant mammals mainly as glucose, fatty acids and triacylglycerols, and amino acids respectively. Their fates in the intermediary metabolism are summarized below (Fig. 13.53).

Carbohydrates

Glucose is transported as the blood sugar to the tissues for mainly energy production.

(a) *Glycolysis*: Considerable amounts of glucose are glycolyzed anaerobically into pyruvate and lactate in the cytoplasm of most tissues. This generates high-energy bonds of ATP through substrate-level phosphorylation (pages 317-322). Glycolysis under aerobic conditions produces far more energy because NADH formed in glycolysis may then contribute its electrons for generating more ATP bonds through oxidative phosphorylation.

(b) *Aerobic oxidation through TCA cycle*: In many tissues including cardiac muscle, liver and kidneys, pyruvate from cytoplasmic glycolysis is oxidatively decarboxylated in mitochondria into acetyl-CoA which is aerobically oxidized in Krebs' TCA cycle to CO_2 and H_2O . This produces a large number of high-energy phosphate bonds through oxidative phosphorylation (pages 326-332).

Besides, the TCA cycle yields intermediates such as succinyl-CoA, citrate and α -ketoglu-

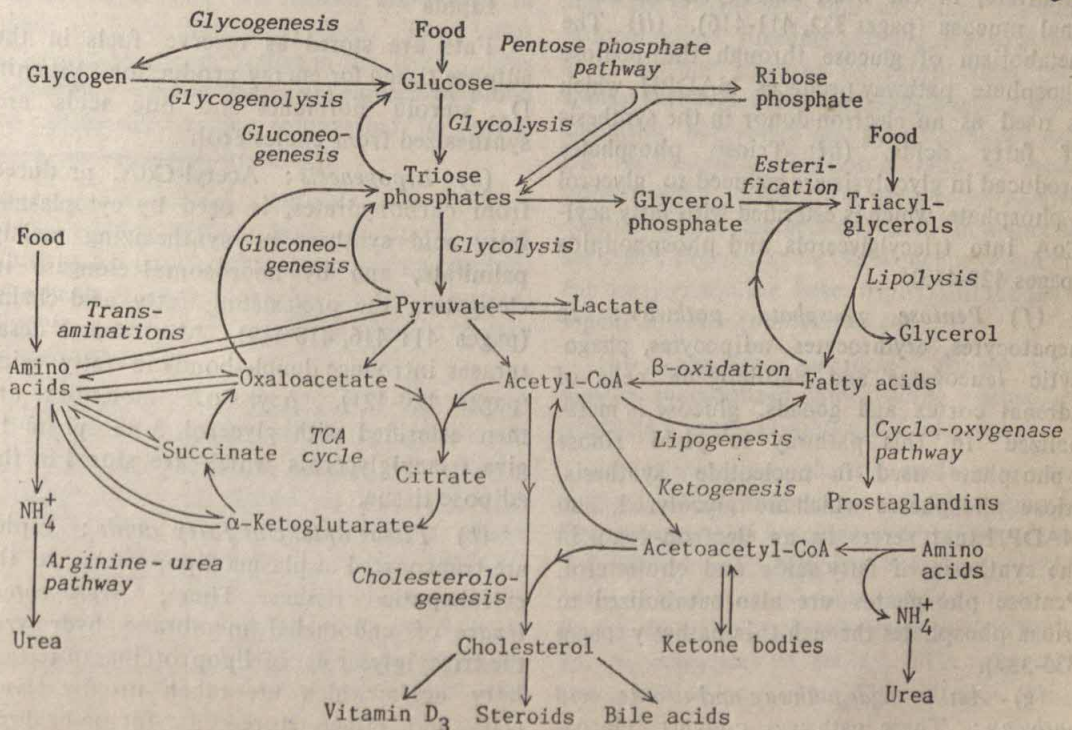


Fig. 13.53. Principal pathways of intermediary metabolism.

tarate which are used in the synthesis of porphyrins, fatty acids and amino acids respectively (page 332).

(c) *Glycogenesis*: In the liver, muscles and other tissues except the brain, erythrocytes and renal cortex, glucose in excess of the immediate need as fuel is largely polymerized by glycogenesis into glycogen which is stored for future use (pages 309-312).

(d) *Glycogenolysis*: Whenever the blood sugar declines, liver glycogen is broken by this process to glucose and released in the blood for supply to the tissues (pages 313-315).

(e) *Lipogenesis*: Glucose in excess of the immediate need is used in synthesizing fat which is stored in adipocytes as a fuel reserve. (i) Acetyl-CoA produced from pyruvate is used by cytoplasmic fatty acid synthase in synthesizing fatty acids, mainly palmitate, in the liver, adipocytes and intestinal mucosa (pages 332, 411-416). (ii) The metabolism of glucose through the pentose phosphate pathway produces NADPH which is used as an electron-donor in the synthesis of fatty acids. (iii) Triose phosphates produced in glycolysis are reduced to glycerol 3-phosphate which is esterified with fatty acyl-CoA into triacylglycerols and phospholipids (pages 429-433).

(f) *Pentose phosphate pathway*: In hepatocytes, erythrocytes, adipocytes, phagocytic leucocytes and steroidogenic cells of adrenal cortex and gonads, glucose is metabolized in this pathway to yield ribose 5-phosphate used in nucleotide synthesis, triose phosphates which are glycolyzed, and NADPH that serves as an electron-donor in the synthesis of fatty acids and cholesterol. Pentose phosphates are also catabolized to triose phosphates through this pathway (pages 335-338).

(g) *Amino sugar pathway and uronic acid pathway*: These pathways convert glucose respectively to hexosamines and uronic acids

for the synthesis of heteroglycans like chondroitin sulfates (pages 349-352).

(h) *Amino acid synthesis*: Carbohydrate metabolites like pyruvate, oxaloacetate and α -ketoglutarate are transaminated into nonessential amino acids such as alanine, aspartate and glutamate (pages 396-398).

(i) *Gluconeogenesis*: Glucose catabolites such as pyruvate and lactate are salvaged by reconversion to glucose in the liver and renal cortex. During carbohydrate deprivation and starvation, glycogenic amino acids like glycine, alanine, serine, aspartate and glutamate are either deaminated or transaminated to give gluconeogenic intermediates like pyruvate, oxaloacetate and α -ketoglutarate, from which glucose is synthesized in the liver and kidneys. Liver releases this glucose in the blood to maintain the blood sugar during starvation (pages 340-345).

Lipids

Fats are stored as reserve fuels in the adipose tissue for energy production. Vitamin D₃, steroid hormones and bile acids are synthesized from cholesterol.

(a) *Lipogenesis*: Acetyl-CoA, produced from carbohydrates, is used by cytoplasmic fatty acid synthase in synthesizing mainly palmitate, and by microsomal elongase in elongating the pre-existing fatty acid chains (pages 411-416, 418-419). Microsomal desaturases introduce double-bonds in fatty acids (pages 420-421). Acyl-CoA molecules are then esterified with glycerol 3-phosphate to give triacylglycerols which are stored in the adipose tissue.

(b) *Tissue uptake of fatty acids*: Lipids are transported as plasma lipoproteins to the extrahepatic tissues. There, *lipoprotein lipase* of endothelial membrane hydrolyzes the triacylglycerols of lipoproteins to release fatty acids which are taken up by tissue cells and either stored as fat or oxidized (page 447).

(c) *Lipolysis*: Whenever necessary, the stored triacylglycerols of adipocytes are hydrolyzed by lipases, including the hormone-sensitive lipase, into fatty acids for oxidation (page 451).

(d) *Beta-oxidation*: In the mitochondrial matrix of the liver, adipose tissue, cardiac muscle and renal cortex, fatty acids undergo beta-oxidation into acetyl-CoA, yielding high-energy bonds of ATP through oxidative phosphorylation (pages 400-404). This is generally followed by the aerobic oxidation of acetyl-CoA in the TCA cycle, producing many more ATP molecules. Fatty acid oxidation supplies much of the energy-need during sustained work, starvation and carbohydrate deprivation.

(e) *Ketogenesis*: In starvation, carbohydrate deprivation and diabetes, acetyl-CoA molecules produced by β -oxidation cannot be oxidized due to the decreased operation of the TCA cycle and are instead condensed in pairs to form acetoacetyl-CoA. The latter gives rise to ketone bodies in the liver (pages 405-408). Ketone bodies are carried by blood to extrahepatic tissues, reconverted to acetyl-CoA and oxidized for energy.

(f) *Cholesterol synthesis*: Acetyl-CoA and acetoacetyl-CoA, both produced from fatty acids, are used in the extramitochondrial synthesis of cholesterol in the liver, adrenal cortex, gonads, skin and intestinal mucosa (pages 439-441).

(g) *Cholesterol metabolism*: (i) Cholesterol is changed to 7-dehydrocholesterol in the skin and intestinal mucosa. 7-dehydrocholesterol is photolyzed in the epidermis by UV rays to precholecalciferol which isomerizes into vitamin D₃ (pages 169-170). (ii) In the adrenocortical and gonadal steroidogenic cells, cholesterol is changed to steroid hormones by hydroxylases and desmolases. (iii) Cholesterol is esterified with fatty acids by *lecithin-cholesterol acyltrans-*

ferase in the plasma and by *acyl-CoA cholesterol acyltransferase* in the extrahepatic tissues. (iv) Cholesterol and its esters are incorporated in lipoproteins and membranes. (v) Cholesterol is catabolized in the liver to bile acids (pages 443-444).

(h) *Prostaglandin synthesis*: C₂₀ polyenoic fatty acids are changed into prostaglandins, thromboxanes and prostacyclin through the *cyclo-oxygenase pathway*, and into leukotrienes through the *lipoxygenase pathway* (pages 423-426).

Proteins

Amino acids are used in synthesizing proteins and various specialized products. The amino-N is removed from amino acids and converted to urea. Amino acids may give rise to glucose and ketone bodies or may be oxidized for energy production.

(a) *Translation of proteins*: Amino acids are polymerized into cytosolic proteins, mitochondrial proteins and exportable as well as membrane proteins by cytoplasmic polyosomes (mRNA-ribosome complexes), mitochondrial polysomes and polysomes on rough ER respectively. Ribosomes help to incorporate amino acids from amino acyl-tRNA complexes into the peptide chain in a specific sequence, depending on the sequence of codons (purine/pyrimidine base triplets) in the participant mRNA (see chapter 14).

(b) *Specialized products*: Amino acids may be metabolized into specific specialized products (pages 372-386 and Table 13.3).

(c) *Nitrogen catabolism and urea formation*: The amino-N is mostly removed from amino acids by either *transamination* or *deamination* and released as NH₄⁺ (pages 357-364). The latter is converted to urea in mammals in the *arginine-urea pathway* with the participation of HCO₃⁻, ATP, aspartate and ornithine (pages 366-368). Urea is excreted in the urine.

TABLE 13.3. *Some specialized products from amino acids.*

Amino acids	Specialized products
Glycine	Creatine, glutathione, heme, purines, C ₁ units, glycocholate, hippurate, glyoxylate
Serine	Sphingosine, phosphatidylserine, C ₁ units
Aspartate	Pyrimidines, purines
Glutamate	Glutathione, γ -aminobutyrate, glutamine
Tyrosine	Catecholamines, melanins, iodothyronins
Tryptophan	Nicotinamide, serotonin, melatonin
Histidine	Histamine
Cysteine	Glutathione, taurine

(d) *Amphibolic products*: The carbon skeletons of amino acids, left after removal of the amino group, are catabolized into gluconeogenic and/or ketogenic products for further metabolism (pages 386-395). According to these amphibolic products, amino acids give rise either to glucose through gluconeogenesis (*glycogenic amino acids*), or to ketone bodies (*ketogenic amino acids*) or to both glucose and ketone bodies (*glycogenic-ketogenic amino acids*).

(e) *Oxidation in TCA cycle*: The gluconeogenic metabolites of amino acids are oxidized in the TCA cycle, producing high-energy bonds of ATP. Acetoacetate from ketogenic

amino acids may be converted to acetyl-CoA in extrahepatic tissues and then oxidized in the TCA cycle. Thus, in starvation and carbohydrate deprivation, amino acids of tissue proteins serve in energy production.

As the metabolites of most intermediary metabolism pathways are ultimately oxidized in the TCA cycle, the latter is considered as the *final common path* of cellular metabolism. Electrons removed from the metabolites in the TCA cycle are transported by the mitochondrial respiratory chain to molecular O₂, yielding the energy for the oxidative phosphorylation of ADP to ATP.

TABLE 13.4. *Amphibolic products from amino acids.*

Amino acids	Amphibolic products
<i>Glycogenic:</i>	
Glycine, alanine, serine, cysteine, hydroxyproline	Pyruvate
Methionine, valine	Succinate
Glutamate, histidine, proline, arginine	α -Ketoglutarate
Aspartate	Oxaloacetate, fumarate
Threonine	Pyruvate, succinate
<i>Ketogenic:</i>	
Lysine, leucine	Acetoacetate
<i>Glycogenic-ketogenic:</i>	
Phenylalanine, tyrosine	Fumarate, acetoacetate
Tryptophan	Pyruvate, acetoacetate
Isoleucine	Succinate, acetate

14. METABOLISM OF NUCLEIC ACIDS

Purine and pyrimidine nucleotides are synthesized mainly from amino acids. They are largely utilized in the synthesis of nucleic acids. The base sequence of a gene of the DNA molecule determines the base sequence of the RNA synthesized on the template of that gene. The base sequence of the mRNA molecule constitutes a genetic code which is translated into the amino acid sequence of the protein synthesized with the help of that mRNA. Each double-helical DNA molecule replicates into two identical DNA molecules.

14.1 Metabolism of pyrimidines

Most of the pyrimidines, used in nucleotide synthesis, are synthesized in the body. Pyrimidine nucleoside triphosphates are used in incorporating pyrimidine nucleotides into RNA and DNA. Some nucleotides such as UDP, CDP and CMP act as carriers of other substrates like hexoses, hexosamines, choline and ethanolamine.

Synthesis

Using isotopically labelled precursors, it has been found that C² of the pyrimidine ring comes from CO₂, N³ from the amide-N of glutamine, and N¹, C⁴, C⁵ and C⁶ respectively from the amino-N, β -carboxyl-C, β -C and α -C of aspartate (Fig. 14.1).

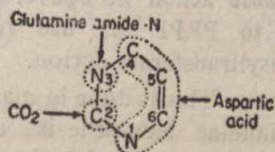


Fig. 14.1. Sources of pyrimidine.

All pyrimidine nucleotides are synthesized through uridine monophosphate or uridyate (UMP) in the body.

(a) The first three enzymes of pyrimidine synthesis, viz., carbamoyl phosphate synthase II, aspartate transcarbamoylase and dihydroorotase, exist in the cytosol as a single multienzyme complex called *complex A* (MW $\approx$ 200 kdal). They act as follows to produce a cyclic compound, dihydroorotic acid, from aspartate, glutamine and CO₂ (Fig. 14.2). (i) *Carbamoyl phosphate synthase II* produces carbamoyl phosphate in the cytosol of liver and other tissues from HCO₃⁻ (i.e., CO₂), the amide-N of glutamine and ATP. This cytoplasmic enzyme, unlike its mitochondrial counterpart for urea biosynthesis, neither requires N-acetylglutamate as a cofactor nor utilizes free NH₄⁺ directly as a substrate; it is allosterically activated by 5-phosphoribosyl 1-pyrophosphate (PRPP) and is inhibited by the feedback effect of UMP and UTP. (ii) *Aspartate transcarbamoylase* (ATC) immediately transfers the carbamoyl group from carbamoyl phosphate to the amino group of L-aspartate to form carbamoylaspartate. This is the rate-limiting step of pyrimidine synthesis. ATC belongs to the K series of allosteric enzymes; it is allosterically activated by ATP and allosterically inhibited by CTP, an endproduct of pyrimidine synthesis. (iii) *Dihydroorotase* next catalyzes a dehydrative closure of the ring in carbamoylaspartate to produce dihydroorotate.

(b) *Dihydroorotate dehydrogenase*, located on the outer surface of the inner mito-

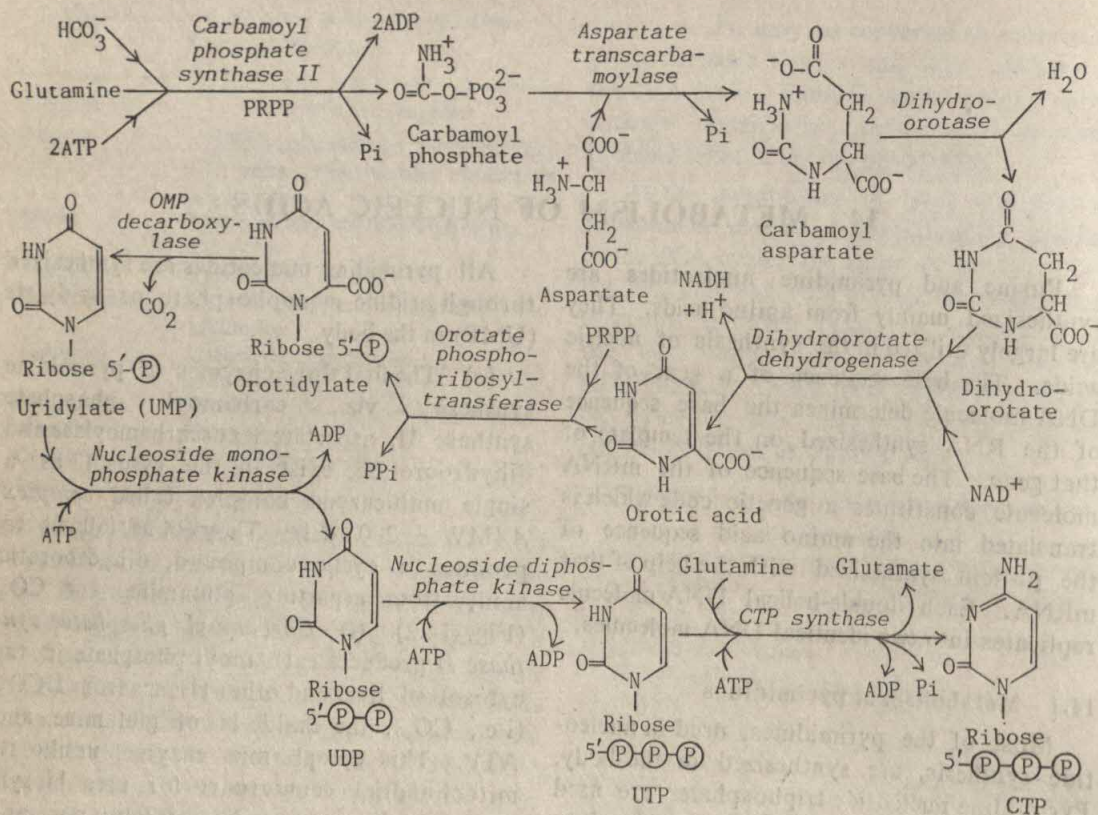


Fig. 14.2. Synthesis of uridylate (UMP), UTP and CTP.

chondrial membrane, oxidizes dihydroorotate to orotic acid by utilizing NAD⁺ as the ultimate electron-acceptor.

(c) The remaining two enzymes of pyrimidine biosynthesis, viz., orotate phosphoribosyltransferase and orotidylate (OMP) decarboxylase, occur in the cytosol as a single multienzyme complex called *complex U*. They convert orotate to uridylate (UMP).

(i) *Orotate phosphoribosyltransferase* transfers the 5-phosphoribosyl group from PRPP to orotate, changing the latter to orotidylate in which orotate is fixed in the β -configuration on the C¹ of ribose. (ii) *OMP decarboxylase* then decarboxylates orotidylate to uridylate (UMP). The decarboxylase is inhibited by the feedback effect of UMP.

The occurrence of the cytosolic enzymes of pyrimidine synthesis as two multienzyme complexes ensures that the intermediates of the pathway are rapidly transferred to the active sites of successive enzymes without diversion to other reactions or dispersal by diffusion in the cytosol.

During pyrimidine (UMP) biosynthesis, two high-energy bonds of ATP are spent in forming carbamoyl phosphate. Two more high-energy phosphate bonds are spent in the *PRPP synthase* action in converting ribose 5-phosphate to PRPP for use in orotate phosphoribosyltransferase action.

Regulation: This is done in many ways.

(a) Pyrimidine biosynthesis is mainly regulated by allosteric modulations of aspar-

tate transcarbamoylase, the rate-limiting enzyme. On binding with its negative allosteric site, UTP and CTP decrease its substrate-affinity and thus inhibit it allosterically to prevent overproduction of pyrimidines. ATP allosterically activates the enzyme to enhance pyrimidine synthesis.

(b) Carbamoyl phosphate synthase II is also allosterically inhibited by the feedback effects of UMP and UTP, but is allosterically activated by PRPP.

(c) Rise in uridylyte concentration inhibits dihydroorotate dehydrogenase and OMP decarboxylase to depress pyrimidine synthesis.

(d) Rise in pyrimidine nucleotide concentration may repress as well as allosterically inhibit PRPP synthase. This reduces pyrimidine synthesis by decreasing the availability of PRPP.

(e) Carbamoylaspartate induces the synthesis of dihydroorotate dehydrogenase and of the protein (complex U) functioning as orotate phosphoribosyltransferase and OMP decarboxylase. It thus enhances pyrimidine synthesis.

Orotic aciduria : (i) Type I orotic aciduria results from an autosomal recessive genetic deficiency of the protein acting as both orotate phosphoribosyltransferase and OMP

decarboxylase. This causes a failure in converting orotate to uridylyte. High blood and urinary levels of orotate, growth retardation and megaloblastic anemia result. (ii) Type II orotic aciduria arises from autosomal recessive genetic deficiency affecting only OMP decarboxylase and is characterized by megaloblastic anemia and the urinary elimination of more orotidine than orotate.

Higher nucleotides of uracil : UMP is phosphorylated to uridine diphosphate (UDP) by ATP and *nucleoside monophosphate kinase* (Fig. 14.2). UDP is further phosphorylated to uridine triphosphate (UTP) by ATP and *nucleoside diphosphate kinase*. UTP is then utilized in synthesizing CTP and RNA.

Cytosine nucleotides : Cytidine triphosphate (CTP) is synthesized from UTP. An amidotransferase called *CTP synthase* utilizes the energy of hydrolysis of ATP to ADP in transferring the amide group of glutamine to the C⁴ of uracil residue in UTP, changing the latter to CTP (Fig. 14.2). CTP is either utilized in RNA synthesis or dephosphorylated by a phosphatase into CDP.

Pseudouridylic acid : This unusual pyrimidine nucleotide is synthesized from uridylyte (UMP) already incorporated in tRNA. A cytoplasmic enzyme shifts the

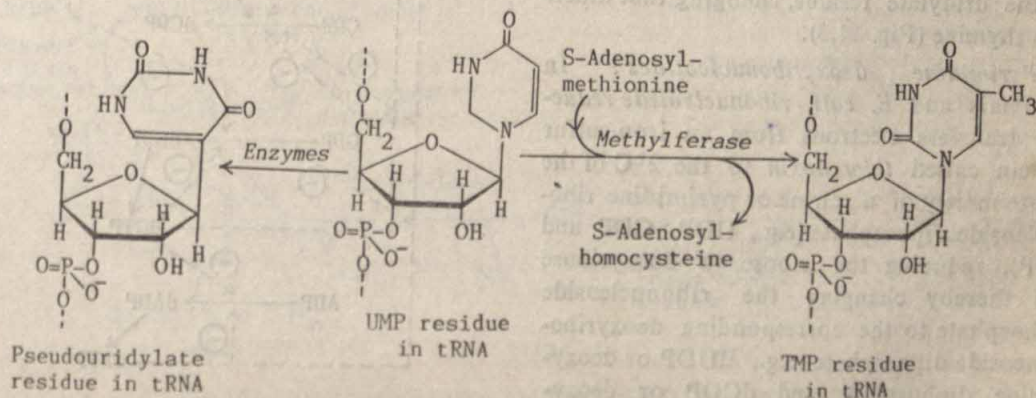


Fig. 14.3. Formation of pseudouridylate and thymidylate (TMP) residues in tRNA from UMP residue.

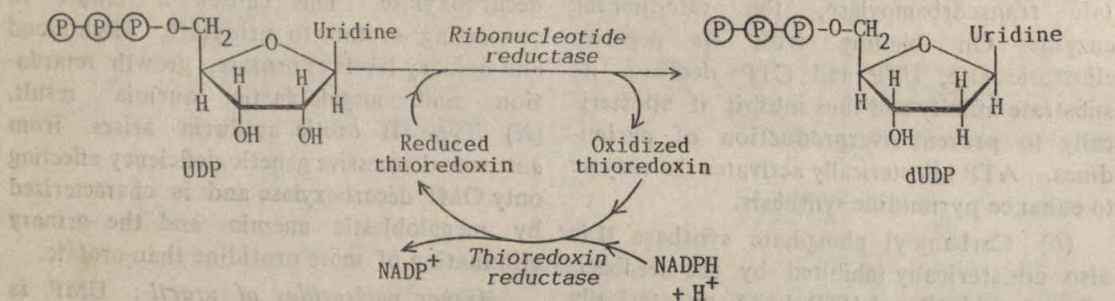


Fig. 14.4. Formation of deoxyribonucleoside diphosphate (dUDP) from ribonucleoside diphosphate (UDP).

phosphoribosyl moiety of UMP from the N^1 to the C^5 of its uracil residue. This changes that uracil nucleoside (uridine) to pseudouridine (ψ); the uridylyte residue of tRNA is thereby converted to a pseudo-uridylyte (Fig. 14.3).

Thymine ribonucleotide: This occurs in the T loop of each tRNA molecule. It is an unusual thymine nucleotide (thymine-ribose-phosphate) because it bears a ribose residue instead of a deoxyribose ordinarily present in thymine nucleotides. Thymine ribonucleotide or thymidylate (TMP) is synthesized from an uridylyte (UMP) residue of tRNA by a *methylferase*. The enzyme transfers the labile methyl group of S-adenosylmethionine to the C^5 of uracil of the uridylyte residue, changing that uracil into thymine (Fig. 14.3).

Pyrimidine deoxyribonucleotides: In mammals and *E. coli*, *ribonucleotide reductase* transfers electrons from an iron-sulfur protein called *thioredoxin* to the 2'-C of the ribose moiety of a purine or pyrimidine ribonucleoside diphosphate (e.g., UDP, CDP and ADP), reducing the ribose to deoxyribose and thereby changing the ribonucleoside diphosphate to the corresponding deoxyribonucleoside diphosphate (e.g., dUDP or deoxyuridine diphosphate and dCDP or deoxycytidine diphosphate). Thioredoxin, oxidized in this reaction, is reduced to its original

form by NADPH and *thioredoxin reductase* having FAD as its prosthetic group (Fig. 14.4).

Ribonucleotide reductase carries non-heme iron in its prosthetic group and requires nucleoside triphosphates as allosteric modulators. For example, on binding with a high-affinity allosteric site of ribonucleotide reductase, ATP promotes its action on UDP and CDP, dGTP enhances the reductase action on ADP but inhibits its action on

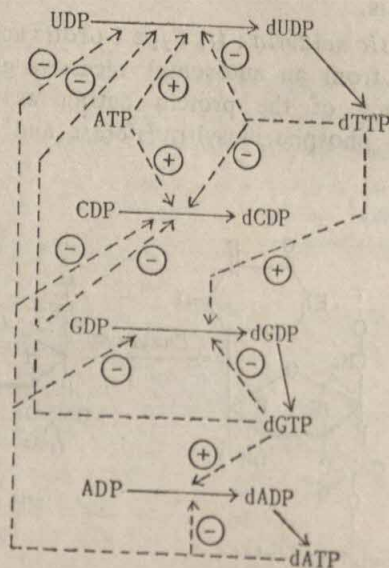


Fig. 14.5. Allosteric modulations (broken lines) of ribonucleotide reductase.

UDP, CIP and GDP while dTTP (deoxythymidine triphosphate) enhances the reductase action on GDP but inhibits its action on CDP and UDP; on the contrary, binding of dATP with a low-affinity allosteric site of the reductase inhibits all its actions (Fig. 14.5). Such allosteric modulations of the reductase maintains a balanced production of deoxyribonucleotides.

Both dCDP and dUDP are utilized in synthesizing deoxythymidylate (deoxythymidine monophosphate or dTMP) whose synthesis is separately described below. dCDP is also phosphorylated by ATP and *nucleoside diphosphate kinase* to produce dCTP for utilization in DNA synthesis.

Unlike the mammalian enzyme, ribonucleotide reductase of *Lactobacillus leichmanii* reduces ribonucleoside triphosphates instead of the diphosphates and requires a cobamide coenzyme instead of thioredoxine.

Thymine deoxyribonucleotides: Deoxythymidylate (dTMP) is synthesized from

dUMP. (i) dUMP may arise from the hydrolysis of dUDP by a *phosphatase*. Alternatively, dCMP may be produced either by the phosphorylation of circulating deoxycytidine with the help of ATP and *deoxycytidine kinase*, or by the hydrolysis of dCDP by a *phosphatase*; dCMP is then deaminated to dUMP by *dCMP deaminase* (Fig. 14.6). (ii) *Thymidylate synthase* then methylates the C⁵ of the uracil residue of dUMP to change the latter to dTMP, utilizing the methylene group of N⁵,N¹⁰-methylene H₄folate—the methylene group is reduced to a methyl group during the transfer while H₄folate is simultaneously oxidized to dihydrofolate (H₂folate). The latter is subsequently reduced back to H₄folate by NADPH and *dihydrofolate reductase*. Both thymidylate synthase and dCMP deaminase are poor in amount in nondividing cells and rise rapidly in activity in dividing cells. (iii) dTMP is subsequently changed to dTDP by phosphorylation with the help of ATP, Mg²⁺ and *thymidylate kinase*. This seems to be

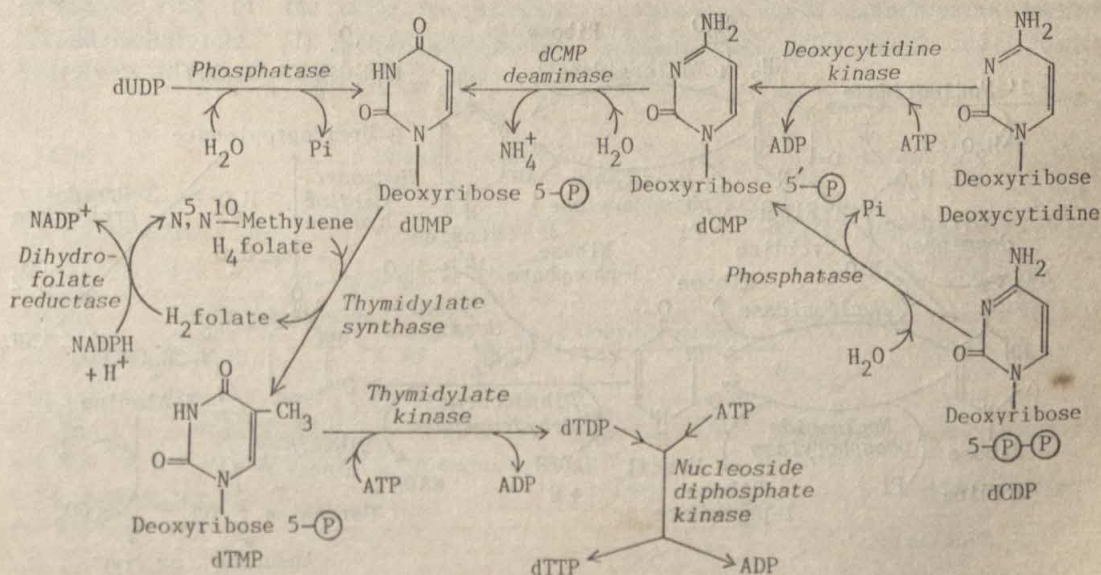


Fig. 14.6. Synthesis of deoxythymidylate (dTMP) and its conversion to dTDP and dTTP.

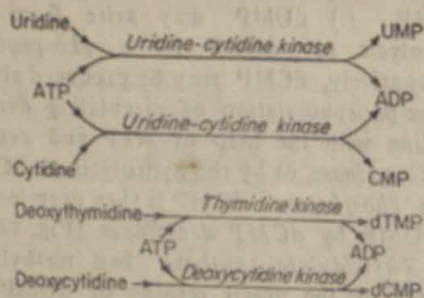


Fig. 14.7. Pyrimidine salvage pathway.

the rate-limiting step in dTTP synthesis. (iv) dTDP is next phosphorylated to dTTP by nucleoside diphosphate kinase, utilizing ATP. dTTP is used in DNA synthesis.

Synthesis of dTMP and so, of DNA is inhibited by folate-antagonists like aminopterin and amethopterin which competitively inhibit dihydrofolate reductase. This explains their therapeutic use in leukemia and other malignancies.

Salvage pathway

Little of the free pyrimidines may be salvaged for utilization in mammalian cells.

But pyrimidine nucleosides may be salvaged by rephosphorylation into respective nucleotides with the help of nucleoside kinases and ATP (Fig. 14.7). Uridine-cytidine kinase rephosphorylates both uridine and cytidine to UMP and CMP respectively; deoxycytidine kinase similarly phosphorylates deoxycytidine to dCMP; thymidine kinase phosphorylates deoxythymidine to dTMP. Thymidine kinase occurs in all dividing cells, phosphorylates deoxyguanosine and deoxyadenosine also to dGMP and dAMP respectively, and is allosterically activated by several deoxyribonucleoside diphosphates, but is allosterically inhibited by dTTP.

Catabolism

Cytosine is catabolized through uracil. (a) Cytidylate (CMP) and deoxycytidylate (dCMP) are first hydrolyzed by 5'-nucleotidase into Pi and respectively cytidine (cytosine 1-ribonucleoside) and deoxycytidine. Cytidine and deoxycytidine are either phosphorylated by nucleoside phosphorylase and Pi into free cytosine and pentose 1-phosphate, or hydro-

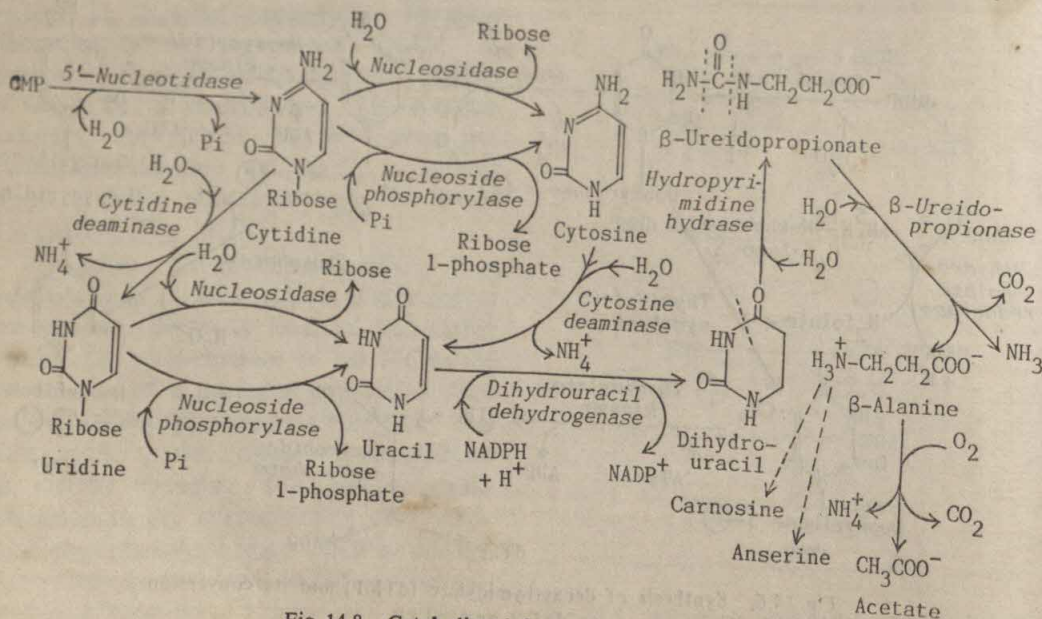


Fig. 14.8. Catabolism of cytosine and uracil.

lyzed by *nucleosidase* into cytosine and pentose. Cytosine is deaminated by *cytosine deaminase* to give uracil (Fig. 14.8); alternatively, cytidine may be directly deaminated by *cytidine deaminase* to give uridine which is then either phosphorylated or hydrolyzed into uracil. (b) *Dihydrouracil dehydrogenase* utilizes NADPH as the electron-donor to reduce uracil to 5,6-dihydrouracil. (c) *Hydropyrimidine hydrase* hydrolytically opens the pyrimidine ring of dihydrouracil to produce β -ureidopropionate. (d) β -Ureido-propionase hydrolyzes the latter into CO_2 , NH_4^+ and β -alanine.

Thymine nucleotides are (a) hydrolyzed by 5'-nucleotidase into either thymidine or deoxythymidine. The product is then either phosphorylated by *nucleoside phosphorylase* or hydrolyzed by *nucleosidase* to give thymine (Fig. 14.9). The latter may also be produced by the deamination of 5-methylcytosine. (b) An NADPH-dependent *dehydrogenase* reduces thymine to dihydrothymine. (c) *Hydropyrimidine hydrase* hydrolytically cleaves the pyrimidine ring of the latter to produce β -ureidoisobutyrate. (d) β -Ureidoisobutyrase hydrolyzes the latter into CO_2 , NH_4^+ and

β -aminoisobutyrate. (e) A *transaminase* transaminates β -aminoisobutyrate into methylmalonic semialdehyde which may give rise to succinate through propionyl-CoA. β -Aminoisobutyrate is also partly excreted in the urine.

Pseudouridylate is hydrolyzed by 5'-nucleotidase into Pi and pseudouridine (uracil 5-ribonucleoside). The latter is then excreted in the urine.

Ammonia produced by pyrimidine catabolism may give rise to urea. But urea from this source is far smaller in amount than that from amino acids.

β -Aminoisobutyric aciduria: This inborn error of pyrimidine catabolism is characterized by high urinary β -aminoisobutyrate and occurs frequently among Oriental Mongolian races. It results from a recessively inherited autosomal genetic error of probably the transaminase involved in the catabolism of β -aminoisobutyrate.

β -Aminoisobutyrate rises in the urine due to increased DNA catabolism in leukemia, tumours, high DNA intake and exposure to X-rays.

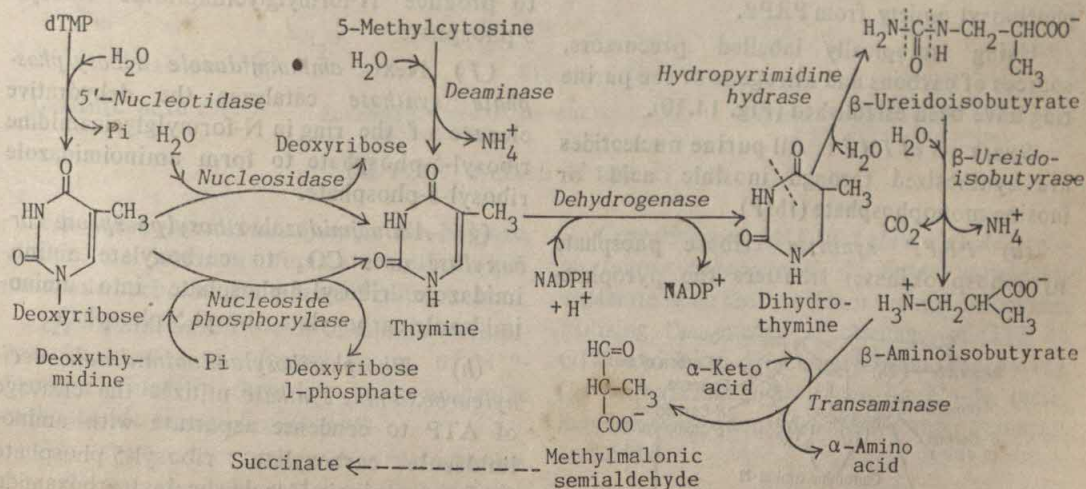


Fig. 14.9. Catabolism of thymine and 5-methylcytosine.

14.2 METABOLISM OF PURINES

Most of the purines, utilized in nucleotide synthesis, are synthesized in the body. Purine nucleoside triphosphates like ATP and GTP serve to incorporate purine nucleotides into nucleic acids and act as energy transducers by storing energy in their high-energy phosphate bonds for utilization in driving endergonic reactions and active transports. GTP is used in the synthesis of tetrahydrobiopterin. GDP acts as a carrier of L-fucose for transglycosylations.

Synthesis

Purine nucleotides can be synthesized by most mammalian tissues including liver and striated muscles. But mature erythrocytes, neutrophils, eosinophils and brain cells possess little or no activity of glutamine PRPP amidotransferase and consequently cannot synthesize purine nucleotides. These tissues depend largely or wholly on the liver for the supply of purine nucleotides. In pyrimidine synthesis, the pyrimidine ring is first synthesized and the phosphoribosyl moiety is then added to it (vide 14.1). But in purine synthesis, the purine ring is gradually built up attached to the pentose residue of a phosphoribosyl moiety from PRPP.

Using isotopically labelled precursors, sources of carbons and nitrogens of the purine ring have been established (Fig. 14.10).

Synthesis of IMP: All purine nucleotides are synthesized through inosinic acid or inosine monophosphate (IMP).

(a) *PRPP synthase* (ribose phosphate pyrophosphokinase) transfers the pyrophos-

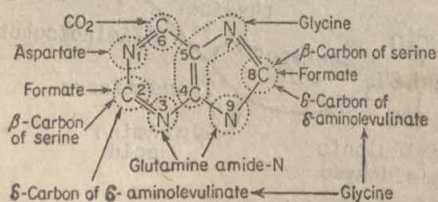


Fig. 14.10. Sources of purine.

phoryl residue of ATP to the C¹ of the ribose residue of ribose 5-phosphate, changing the latter to 5-phosphoribosyl 1-pyrophosphate (PRPP) (Fig. 14.11). The latter serves as the phosphoribosyl donor for the synthesis of purines, pyrimidines, NAD⁺ and NADP⁺ and also for purine salvage pathways.

(b) *Glutamine PRPP amidotransferase* transfers the amide group of glutamine to the C¹ of PRPP, releasing its pyrophosphate group as P_i and forming 5-phosphoribosyl-1-amine. There is a simultaneous inversion of the C¹-substituent group of ribose from α to β configuration.

(c) *Glycinamide kinosynthase* next condenses glycine with 5-phosphoribosyl 1-amine, utilizing the energy of hydrolysis of an ATP to ADP and P_i. This produces 5-phosphoribosylglycinamide (glycinamide ribotide).

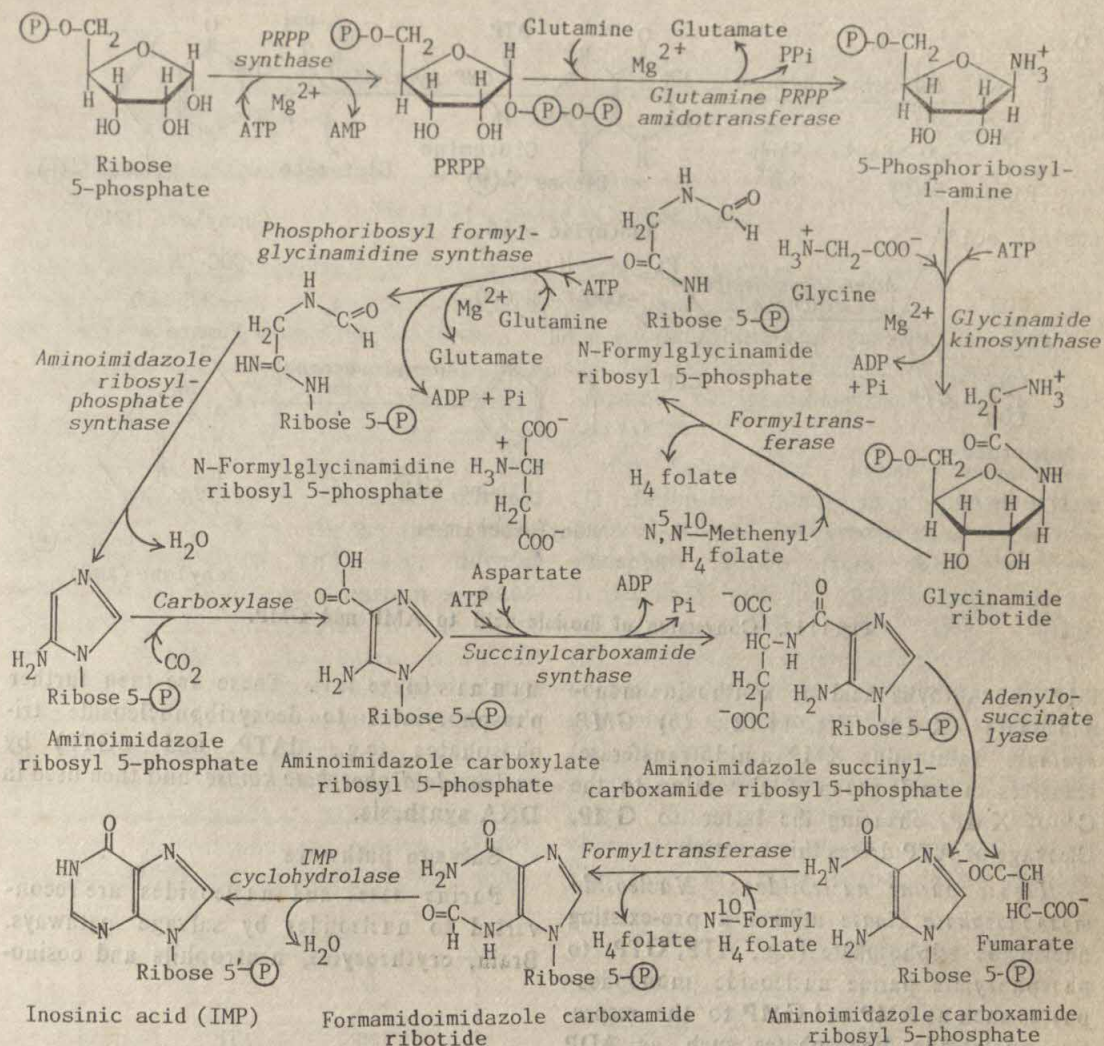
(d) *Phosphoribosylglycinamide formyltransferase* next uses the methenyl group of N⁵,N¹⁰-methenyl-H₄ folate to formylate the amino-N of glycinamide ribotide, forming N-formylglycinamide ribosyl-5-phosphate.

(e) *Phosphoribosylformylglycinamide synthase* transfers another amide group of glutamine to N-formylglycinamide ribotide to produce N-formylglycinamide ribosyl-5-phosphate.

(f) Next, *aminoimidazole ribosylphosphate synthase* catalyzes the dehydrative closure of the ring in N-formylglycinamide ribosyl-5-phosphate to form aminoimidazole ribosyl-5-phosphate.

(g) *Aminoimidazole ribosylphosphate carboxylase* uses CO₂ to carboxylate aminoimidazole ribosyl-5-phosphate into aminoimidazole carboxylate ribosyl-5-phosphate.

(h) *Phosphoribosylaminoimidazole-succinylcarboxamide synthase* utilizes the cleavage of ATP to condense aspartate with aminoimidazole carboxylate ribosyl-5-phosphate to form aminoimidazole succinylcarboxamide ribosyl-5-phosphate.



(i) *Adenylosuccinate lyase*, a C—N lyase, cleaves the latter to fumarate and aminoimidazole carboxamide ribosyl-5-phosphate.

(j) *Aminoimidazole carboxamide formyl-transferase* uses the formyl group of N¹⁰-formyl H₄ folate to formylate aminoimidazole carboxamide ribosyl-5-phosphate.

(k) The product undergoes a dehydrative ring closure by *IMP cyclohydrolase* to form inosinic acid (IMP).

3. Conversion of IMP to AMP : (a) *Adenylosuccinate synthase*, a C—N ligase, condenses aspartate with IMP to form adenylosuccinate, utilizing the energy of cleavage of GTP to GDP and Pi to drive the reaction (Fig. 14.12). (b) *Adenylosuccinate lyase*, a C—N lyase, then cleaves adenylosuccinate into fumarate and AMP.

Conversion of IMP to GMP: (a) IMP dehydrogenase uses NAD^+ and H_2O to oxidize

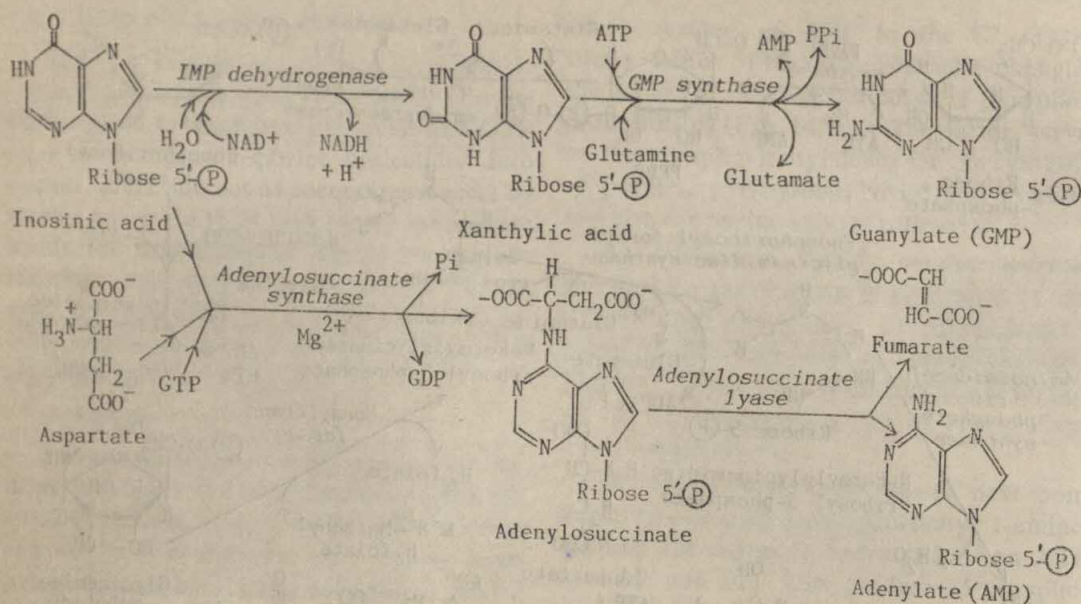


Fig. 14.12. Conversion of inosinic acid to AMP and GMP.

IMP to xanthylic acid or xanthosine monophosphate (XMP) (Fig. 14.12). (b) *GMP synthase* (glutamine XMP amidotransferase) transfers an amide group of glutamine to the C² of XMP, changing the latter to GMP. Cleavage of ATP drives this reaction.

Higher purine nucleotides: *Nucleoside monophosphate kinase* utilizes a pre-existing nucleoside triphosphate (e.g., ATP, GTP) to phosphorylate purine nucleoside monophosphates such as AMP and GMP to the respective nucleoside diphosphates such as ADP and GDP (Fig. 14.13). Similarly, *nucleoside diphosphate kinase* phosphorylates nucleoside diphosphates like ADP and GDP to the respective nucleoside triphosphates with the help of pre-existing nucleoside triphosphates.

Purine deoxyribonucleotides: As in the case of pyrimidines, purine ribonucleoside diphosphates (e.g., ADP and GDP) are reduced to respective purine deoxyribonucleoside diphosphates (e.g., dADP and dGDP) by *ribonucleotide reductase* with the help of *thioredoxin* and *thioredoxin reductase* in

minutis (page 464). These are then further phosphorylated to deoxyribonucleoside triphosphates (e.g., dATP and dGTP) by *nucleoside diphosphate kinase* and then used in DNA synthesis.

Salvage pathways

Purine bases and nucleosides are reconverted to nucleotides by salvage pathways. Brain, erythrocytes, neutrophils and eosino-

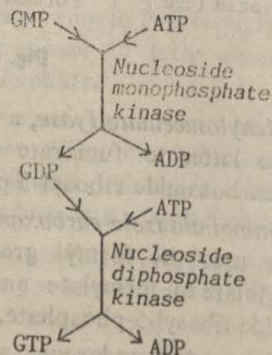
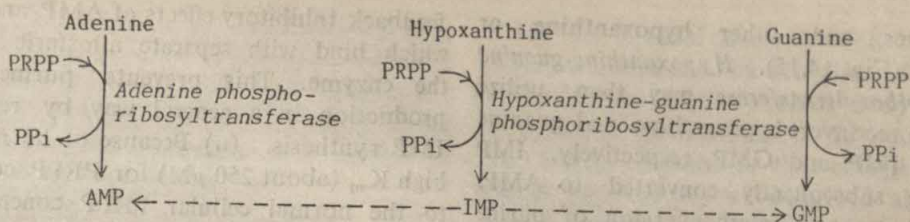


Fig. 14.13. Phosphorylation of GMP to GDP and GTP.

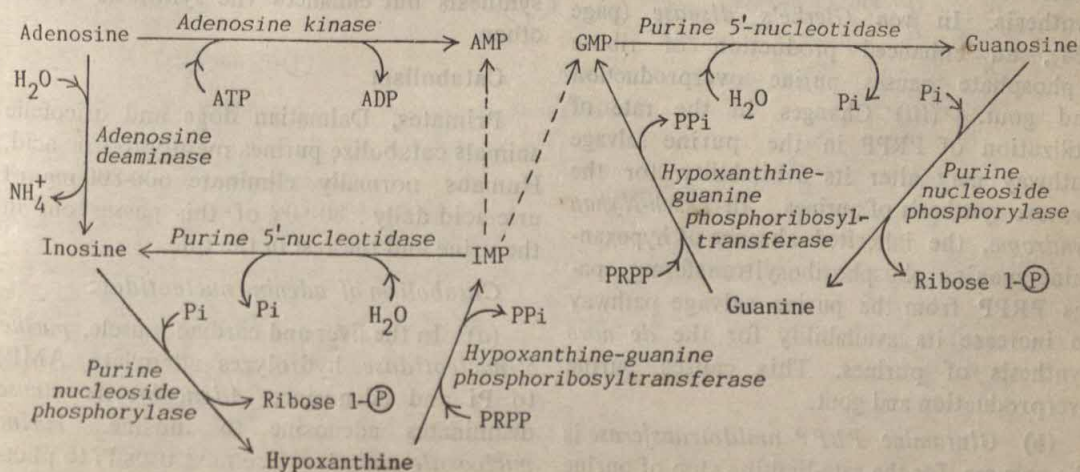


phils produce most of their purine nucleotides by the salvage of purine bases and nucleosides, reaching them from the liver.

(a) *Salvage of purine bases*: (i) On feeding rats and pigeons with ^{15}N -adenine, significant amounts of ^{15}N -adenine residues appear in their tissue nucleotides and nucleic acids. Thus, adenine may be directly salvaged for nucleotide synthesis. In the liver, blood cells, kidneys, brain and skin, *adenine phosphoribosyltransferase* transfers a phosphoribosyl group from PRPP to the N^9 of adenine, changing the latter to AMP (Fig. 14.14). (ii) A far more active salvage enzyme called *hypoxanthine-guanine phosphoribosyltransferase* transfers the phosphoribosyl group from PRPP to the N^9 of hypoxanthine, guanine or xanthine, converting them respectively to inosinic (IMP), guanylic (GMP) and xanthylic (XMP) acids.

IMP and XMP are then used in synthesizing AMP and GMP (Fig. 14.12). The salvage of hypoxanthine and guanine is regulated by the feedback inhibition of hypoxanthine-guanine phosphoribosyltransferase by IMP and GMP.

(b) *Salvage of purine nucleosides:*
(i) *Adenosine kinase* may phosphorylate adenosine and deoxyadenosine with a phosphate group from ATP to form respectively adenylic (AMP) and deoxyadenylic (dAMP) acids (Fig. 14.15).
(ii) *Adenosine deaminase* can deaminate some adenosine to inosine; moreover, inosine, guanosine, deoxyinosine and deoxyguanosine may be formed by the hydrolysis of the respective nucleotides by *purine 5'-nucleotidase*. These purine nucleosides may then be phosphorylated by *purine nucleoside phosphorylase* in most tissues into pentose



phosphates and either hypoxanthine or guanine (Fig. 14.15). *Hypoxanthine-guanine phosphoribosyltransferase* may then utilize PRPP to reconvert hypoxanthine and guanine back to IMP and GMP respectively. IMP may be subsequently converted to AMP. This salvage and interconversion of purine nucleosides constitutes the *purine salvage cycle*. (iii) *Deoxycytidine kinase*, a pyrimidine salvage enzyme, may phosphorylate deoxyadenosine and deoxyguanosine with a phosphate group from ATP to form respectively dAMP and dGMP.

Regulation of purine synthesis

(a) The *de novo* synthesis of purines is largely regulated through the availability of PRPP. (i) *PRPP synthase* is allosterically inhibited by the feedback effects of PRPP and a number of purine nucleotides such as AMP, GMP, ADP, GDP, NAD^+ and FAD. Such inhibition of PRPP synthesis decreases purine synthesis. Inherited defects of PRPP synthase may enhance its activity by either increasing its V_{max} , or making it feedback-resistant, or increasing its substrate-affinity for ribose 5-phosphate. All such cases result in purine overproduction, leading to gout. (ii) Increased availability of ribose 5-phosphate for PRPP synthesis enhances purine synthesis. In *von Gierke's disease* (page 334), an enhanced production of ribose 5-phosphate causes purine overproduction and gout. (iii) Changes in the rate of utilization of PRPP in the purine salvage pathway may alter its availability for the *de novo* synthesis of purines. In *Lesch-Nyhan syndrome*, the inherited absence of hypoxanthine-guanine phosphoribosyltransferase spares PRPP from the purine salvage pathway to increase its availability for the *de novo* synthesis of purines. This causes purine overproduction and gout.

(b) *Glutamine PRPP amidotransferase* is the enzyme for the rate-limiting step of purine synthesis. (i) It is largely regulated by the

feedback inhibitory effects of AMP and GMP which bind with separate allosteric sites of the enzyme. This prevents purine overproduction in a general way by regulating IMP synthesis. (ii) Because of its relatively high K_m (about $250 \mu\text{M}$) for PRPP compared to the normal cellular PRPP concentration (about $40\text{--}50 \mu\text{M}$), the amidotransferase activity depends largely on the availability of PRPP. Moreover, the sigmoidal kinetics of the enzyme ensures a considerable rise in its activity with a small rise in PRPP concentration. (iii) Because of its relatively high K_m for glutamine, the enzyme activity is considerably increased with the rise in glutamine availability also.

(c) A balance is maintained between adenine and guanine nucleotides by regulating the post-IMP branches of the pathway. (i) AMP and GMP cause endproduct feedback inhibition of *adenylosuccinate synthase* and *IMP dehydrogenase* respectively. Thus, each nucleotide prevents its own overproduction by inhibiting its own synthesis from IMP. (ii) In the conversion of IMP to AMP, *adenylosuccinate synthase* requires GTP; in the pathway from IMP to GMP, *GMP synthase* requires ATP. These ensure that an excess of one purine nucleotide reduces its own synthesis but enhances the synthesis of the other.

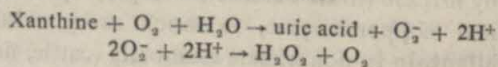
Catabolism

Primates, Dalmatian dogs and uricotelic animals catabolize purines mainly to uric acid. Humans normally eliminate 600–800 mg of uric acid daily; 80–90% of this passes out in the urine and the rest in the bile.

Catabolism of adenine nucleotides:

(a) In the liver and cardiac muscle, *purine 5'-nucleotidase* hydrolyzes adenylylate (AMP) to Pi and adenosine. *Adenosine deaminase* deaminates adenosine to inosine. *Purine nucleoside phosphorylase* next uses Pi to phosphorylate inosine to ribose 1-phosphate and

hypoxanthine (Fig. 14.16). *Xanthine oxidase*, a metalloflavoprotein, oxidizes hypoxanthine to xanthine, and xanthine to uric acid; molecular O_2 is reduced at each stage to the superoxide anion O_2^- which is converted to H_2O_2 by *superoxide dismutase*.



(b) In skeletal muscles, some adenylate (AMP) is deaminated by *adenylate deaminase* to inosinic acid (IMP) which is hydrolyzed by *purine 5'-nucleotidase* to P_i and inosine (Fig. 14.16). Inosine is then changed to uric acid in the same way as described above. A rise in cellular GMP level inhibits adenylate deaminase to reduce the catabolism of AMP

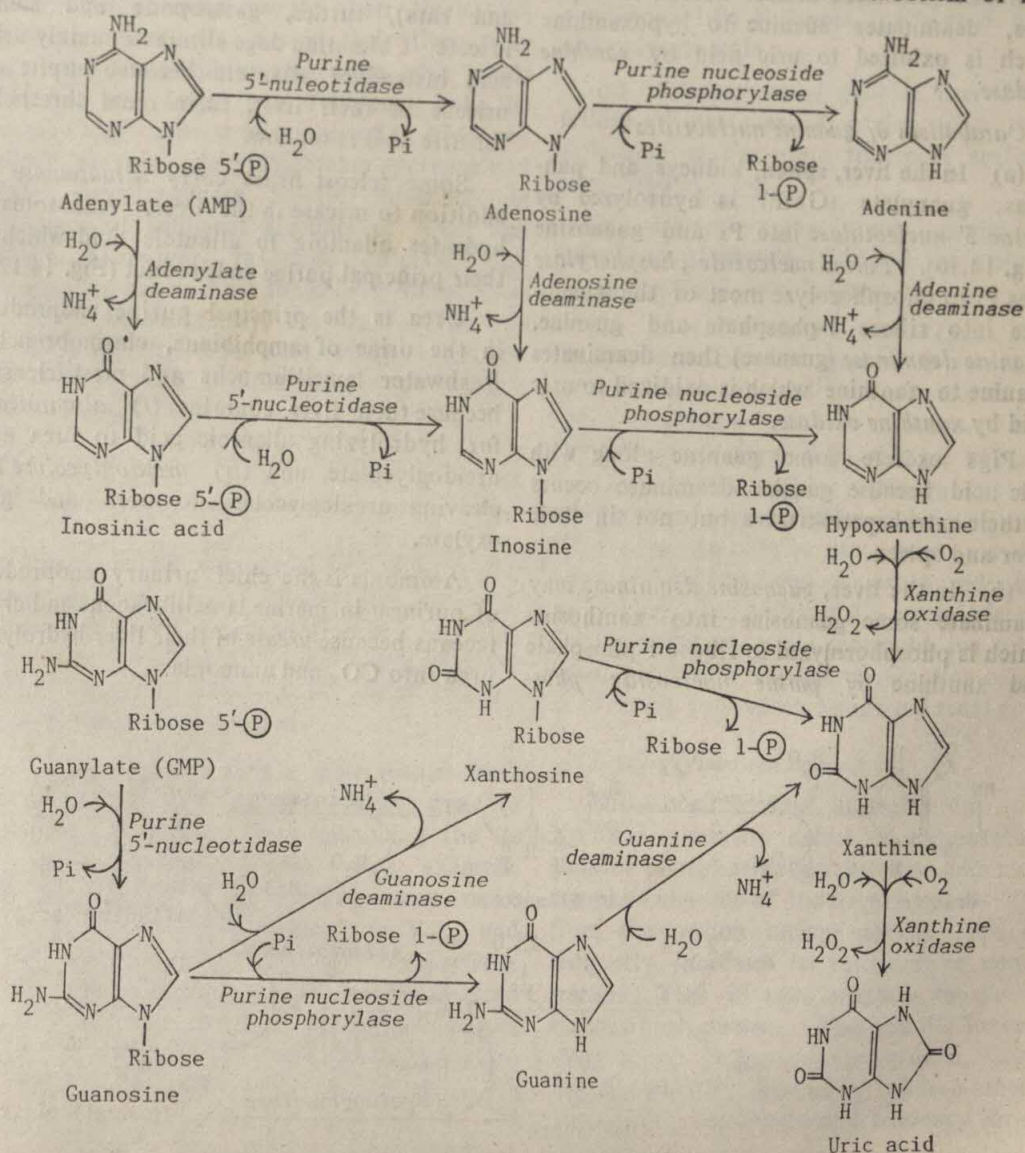


Fig. 14.16. Catabolism of purines to uric acid.

and thus helps to maintain a balance between adenine and guanine nucleotides.

(c) In invertebrates and bacteria, AMP is hydrolyzed by *purine 5'-nucleotidase* into Pi and adenosine. *Purine nucleoside phosphorylase* then phosphorylates adenosine to adenine and ribose 1-phosphate. *Adenine deaminase* (adenase), almost absent in mammals, deaminates adenine to hypoxanthine which is oxidized to uric acid by *xanthine oxidase*.

Catabolism of guanine nucleotides :

(a) In the liver, spleen, kidneys and pancreas, guanylate (GMP) is hydrolyzed by *purine 5'-nucleotidase* into Pi and guanosine (Fig. 14.16). *Purine nucleoside phosphorylase* uses Pi to phosphorylate most of the guanosine into ribose 1-phosphate and guanine. *Guanine deaminase* (guanase) then deaminates guanine to xanthine which is oxidized to uric acid by *xanthine oxidase*.

Pigs excrete some guanine along with uric acid because guanine deaminase occurs in their extrahepatic tissues but not in their liver and spleen.

(b) In the liver, *guanosine deaminase* may deaminate some guanosine into xanthosine which is phosphorylated to ribose 1-phosphate and xanthine by *purine nucleoside phos-*

phorylase. Xanthine is then oxidized to uric acid by *xanthine oxidase*.

Uric acid catabolism :

Uric acid is oxidized and decarboxylated by *uricase* (urate oxidase), a hepatic peroxisomal copper-enzyme, to much more soluble allantoin in nonprimate mammals (cattle, dogs and rats), turtles, gastropods and some insects. Camelion dogs eliminate mainly uric acid instead of allantoin because in spite of *uricase* in their liver, their renal threshold for uric acid is too low.

Some teleost fishes carry *allantoinase* in addition to *uricase* in the liver. Allantoinase hydrates allantoin to allantoinic acid which is their principal purine endproduct (Fig. 14.17).

Urea is the principal purine endproduct in the urine of amphibians, elasmobranchs, freshwater lamellibranchs and most teleosts, because their liver contains (i) *allantoicase* for hydrolyzing allantoinic acid to urea and ureidoglycolate, and (ii) *ureidoglycolase* for cleaving ureidoglycolate to urea and glyoxylate.

Ammonia is the chief urinary endproduct of purines in marine lamellibranchs and crustaceans because *urease* of their liver hydrolyzes urea into CO_2 and ammonia.

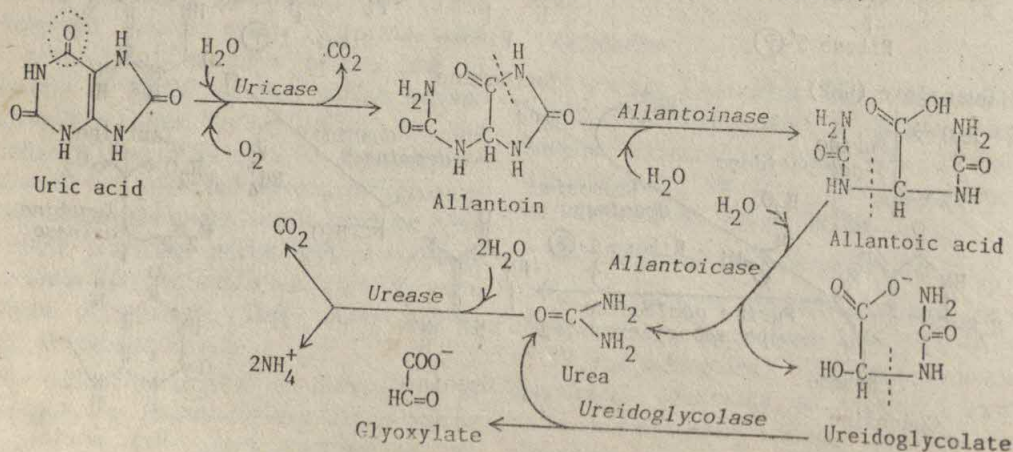


Fig. 14.17. Catabolism of uric acid in different animals.

Purine metabolism in birds

In *uricotelic animals* like birds, purine nucleotides have far weaker inhibitory effects on glutamine PRPP amidotransferase than their regulatory actions on adenylosuccinate synthase and IMP dehydrogenase; on the contrary, high protein intakes enhance the activities of glutamine synthase and glutamine PRPP amidotransferase in their liver. So, IMP synthesis continues in their liver from protein endproducts even when the needs for purines have been fulfilled; but the excess IMP cannot be converted to AMP and GMP because adenylosuccinate synthase and IMP dehydrogenase remain inhibited in presence of sufficient amounts of AMP and GMP. Moreover, in these animals, high protein intakes enhance the activity of xanthine oxidase. Consequently, the excess IMP is catabolized to uric acid which eliminates the nitrogen from protein catabolism in their urine (page 365).

Clinical disorders

Gout :

Gout is characterized by an abnormal rise in serum uric acid (*hyperuricemia*), deposition of urate crystals at joints, under the skin and in soft tissues and cartilages, inflammation and swelling at joints, urate calculi in renal tubules, and renal damages.

(a) *Primary metabolic gout* results from an overproduction of uric acid due to X-linked recessive defects enhancing the *de novo* synthesis of purines. For example, such defects of PRPP synthase may make it feedback resistant, increase its V_{max} and lower its K_m for ribose 5-phosphate. PRPP synthase activity is thereby enhanced to increase purine synthesis. X-linked recessive defects of hypoxanthine-guanine phosphoribosyltransferase may reduce the utilization of PRPP in the purine salvage pathway, leading to an increased intracellular

concentration of PRPP. This in turn enhances the *de novo* purine synthesis (see *Lesch-Nyhan syndrome*). On the contrary, a genetic defect of xanthine oxidase may make it hyperactive and thus enhance the catabolism of purines to uric acid, producing gout.

(b) *Secondary metabolic gout* results from a secondary increase in purine catabolism in conditions like leukemia, prolonged fasting and polycythemia.

(c) *Primary renal gout* is caused by a failure of urate excretion due to a genetic deficiency of the urate transport system in renal tubules.

(d) *Secondary renal gout* results from a failure of glomerular filtration of urate due to a generalized renal failure.

(e) In *von Gierke's disease* (page 334), a genetic deficiency of glucose 6-phosphatase leads to a heightened rate of pentose phosphate pathway and a consequent overproduction of ribose 5-phosphate, the substrate for PRPP synthase; this enhances the synthesis of purines followed by their catabolism, to produce a secondary metabolic gout. Simultaneously, a prolonged lactic acidosis decreases the renal urate clearance by increasing the renal threshold for tubular urate secretion; this causes retention of urate in the body to produce a primary renal gout.

Lesch-Nyhan syndrome :

This occurs only in males due to an X-linked, recessive defect of hypoxanthine-guanine phosphoribosyltransferase. An almost complete absence of the enzyme spares PRPP from the purine salvage pathway and consequently increases its intracellular concentration. This in turn enhances the *de novo* synthesis of purines. This results in severe gout, renal failure, poor growth, mental retardation, cerebral palsy, choreo-athetotic movements, spasticity and tendency for self-mutilation.

Xanthinuria :

An autosomal recessive deficiency of xanthine oxidase blocks the oxidation of hypoxanthine and xanthine to uric acid. This produces high urinary levels of xanthine and hypoxanthine, xanthine lithiasis and low serum urate (*hypouricemia*).

Adenosine deaminase deficiency :

An autosomal recessive deficiency of adenosine deaminase causes the urinary elimination of deoxyadenosine, depopulation and functional failure of lymphocytes and a severe immunodeficiency affecting cell-mediated as well as humoral immunity.

Nucleoside phosphorylase deficiency :

An autosomal recessive deficiency of purine nucleoside phosphorylase causes the urinary elimination of hypoxanthine and guanine nucleosides, reduced production and elimination of uric acid, and a severe deficiency of cell-mediated immunity.

14.3 SYNTHESIS OF TETRAHYDRO-BIOPTERIN

Tetrahydrobiopterin functions as the electron-donor coenzyme for phenylalanine

hydroxylase, tyrosine hydroxylase and tryptophan hydroxylase.

Guanosine triphosphate (GTP) is converted by *GTP cyclohydrolase* into formate and dihydroneopterin triphosphate (Fig. 14.18). The latter is changed to sepiapterin through dephosphorylations. *Sepiapterin reductase* uses NADPH as the electron-donor to reduce sepiapterin to dihydrobiopterin. *Dihydrobiopterin reductase* utilizes NADPH to reduce dihydrobiopterin to tetrahydrobiopterin.

Genetic deficiency of GTP cyclohydrolase produces tetrahydrobiopterin deficiency and thereby blocks the normal metabolism of phenylalanine through its hydroxylation to tyrosine. This may cause *phenylketonuria*, a fatal inborn error of phenylalanine metabolism (page 389).

14.4 REPLICATION OF DNA

Replication of DNA is the synthesis of a new DNA molecule by the polymerization of deoxyribonucleotides, using a pre-existing DNA molecule as the template. The two strands of a pre-existing DNA double-helix progressively unwind and separate for a short stretch at a time from each other with the breakage of hydrogen bonds between them

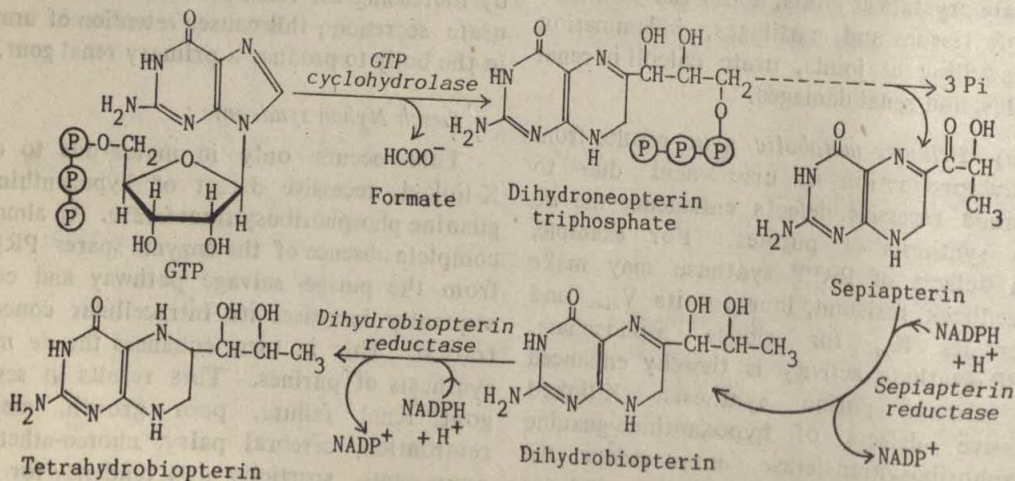


Fig. 14.18. Synthesis of tetrahydrobiopterin.

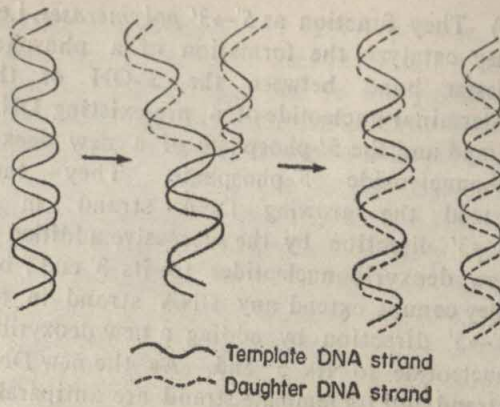


Fig. 14.19. Semiconservative replication.

(Fig. 14.19). Each such strand acts as a template strand and a new DNA strand is laid down against its unwound segment. As the unwinding spreads over the rest of the DNA duplex, the laying down of the new DNA strand extends progressively over the rest of each template strand. Each daughter strand, thus synthesized, is complementary in base sequence to its template strand and winds with the latter to form a new DNA double-helix. The original DNA double-helix thus gets replaced by two new DNA double-helices, each consisting of a pre-existing template strand and a newly-laid daughter strand. This mode of replication is called *semiconservative*.

Nuclear DNA is replicated in animals during the synthetic or *S* phase of the cell cycle. After the preceding mitosis or *M* phase, there is a relatively long nonsynthetic phase called gap-1 or *G*₁ phase which is followed by the *S* phase. Another relatively short nonsynthetic gap-2 or *G*₂ phase intervenes between the *S* phase and the next *M* phase.

Unwinding of DNA

For replication, both strands of the pre-existing DNA double-helix act as templates for the polymerization of the respective daughter strands. This needs the exposure of

the bases of template strands by their unwinding from each other. In eukaryotes, replication starts with the unwinding of the DNA double-helix at multiple specific points distanced by about 30 μ m from each other (Fig. 14.20). At each such starting point, the separation of the two strands produces a *replication bubble* with two fork-like ends called *replication forks*. Each fork moves away from that at the other end of the bubble. Thus, the unwinding followed by replication spreads in both directions from each replication bubble and over the double-stranded regions of DNA intervening between successive bubbles (*bidirectional replication*).

Specific *DNA unwinding proteins* or *DNA helicases* effect the unwinding and local separation of the template strands. The helicase binds with the DNA double-helix, separates the base-pairs of the two strands with the energy of hydrolysis of ATP, and thereby unwinds the strands to create a replication fork (Fig. 14.21). As the helicase travels down the DNA double-helix, it progressively unwinds the strands of the latter and makes the replication fork travel in the direction of progress of the replication.

Several molecules of a *single-stranded DNA binding protein* or *helix destabilizing (HD) protein* bind stoichiometrically with each of the separated single-strands of the DNA to stabilize it for serving as a template for replication. As the replication fork advances, HD protein molecules dissociate from the

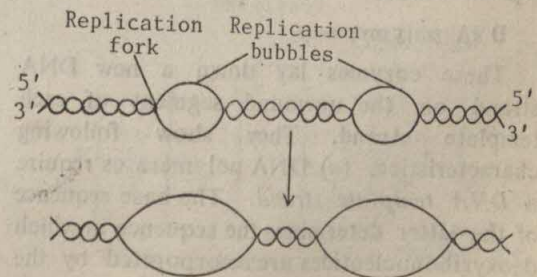


Fig. 14.20. Bidirectional replication.

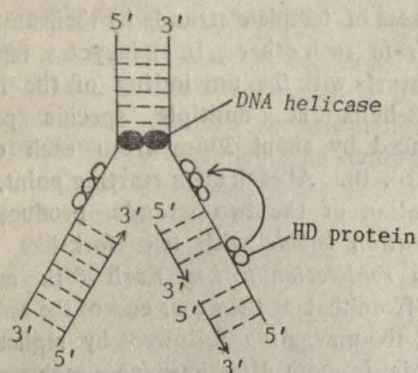


Fig. 14.21. Action of DNA helicase and HD protein at a replication fork.

strand and go to bind at more advanced sites on the latter.

The progressive unwinding of the two DNA strands results in a torque. This is relieved by a rotation of the strands at about $10^4 \text{ rev min}^{-1}$ with the help of a *DNA swivelase*, also called *DNA topoisomerase I* or *nicking-closing enzyme*. This enzyme hydrolyzes a 5',3'-phosphodiester bond in the backbone of one template strand and binds by a high-energy bond to the phosphorylated 5' end of one of its cut segments, utilizing the energy generated in the DNA double-helix by its unwinding (Fig. 14.22). The two ends of the nick (cut) then rotate freely to untwist the helical turns and relieve the torque. The nick is next resealed by a 5',3'-phosphodiester bond between the nicked terminals of the DNA strand, utilizing the energy of cleavage of the bond between the nicked strand and the topoisomerase.

DNA polymerases

These enzymes lay down a new DNA strand on the unwound segment of each template strand. They show following characteristics. (a) DNA polymerases require *DNA template strand*. The base sequence of the latter determines the sequence in which deoxyribonucleotides are incorporated by the polymerase in the growing DNA strand.

(b) They function as 5'→3' polymerases, i.e., they catalyze the formation of a phosphodiester bond between the 3'-OH of the 3'-terminal nucleotide of a pre-existing DNA strand and the 5'-phosphate of a new deoxyribonucleoside 5'-phosphate. They thus extend the growing DNA strand in a 5'→3' direction by the successive addition of new deoxyribonucleotides to its 3' end; but they cannot extend any DNA strand in the 3'→5' direction by adding a new deoxyribonucleotide to its 5' end. As the new DNA strand and its template strand are antiparallel with the 3' end of one facing the 5' end of the other and vice versa, DNA polymerases copy the template strand in the 3'→5' direction only. (c) DNA polymerases cannot directly initiate DNA synthesis; instead, they start laying down a DNA strand by adding the first deoxyribonucleotide to the 3' end of an already-laid *RNA primer strand* of about 10-200 ribonucleotides, hydrogen-bonded to the complementary bases of the DNA template strand. (d) DNA polymerases require the presence of *all four deoxyribonucleoside 5'-triphosphates*, viz., dATP, dGTP, dCTP and dTTP, which they utilize as substrates for incorporating new deoxyribonucleotides in the growing DNA strand.

Eukaryotic cells such as hepatocytes, fibroblasts and thymocytes possess three types

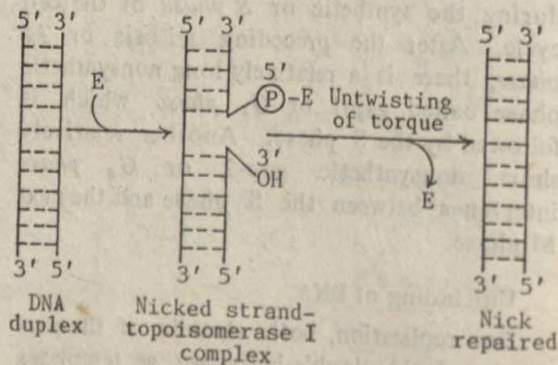


Fig. 14.22. Nick-sealing action of DNA topoisomerase I. E: Enzyme

of DNA polymerases. (i) *DNA polymerase α* occurs in the nucleus, rises in amount in the S phase of the cell cycle and is the principal enzyme for the replication of nuclear DNA. Its core enzyme has an MW of about 156 kdal in rat and forms an active hetero-oligomeric holoenzyme in association with several smaller subunits. It has no nuclease activity. (ii) *DNA polymerase β* (MW 43 kdal) also occurs in the nucleus, does not increase in amount in the S phase and participates mainly in the repair of nuclear DNA. (iii) *DNA polymerase γ* (MW 119 kdal) occurs in mitochondria and catalyzes the replication of mitochondrial DNA.

E. coli has three types of DNA polymerases. (i) *DNA polymerase I* consists of a single peptide chain (MW 109 kdal) and has 5'→3' polymerase, 5'→3' exonuclease and 3'→5' exonuclease activities. As 3'→5' exonuclease, it removes hydrolytically a mismatched deoxyribonucleotide from the 3' end of a DNA strand (*proof-reading action*). As 5'→3' exonuclease, it removes an RNA primer or a faulty DNA strand lying in the path of a growing DNA strand, by hydrolyzing its phosphodiester bonds serially in the 5'→3' direction starting from its 5' end (Fig. 14.23).

This polymerase, also called *Kornberg enzyme*, participates mainly in the repair of DNA; it also removes mismatched bases, RNA primers and faulty DNA strands and fills the resulting gaps by laying down fresh segments of DNA strands. (ii) *DNA polymerase II* (MW 120 kdal) functions as 5'→3' polymerase and 3'→5' exonuclease, but lacks 5'→3' exonuclease activity. (iii) *DNA polymerase III* exists as a multienzyme complex in the active state. It possesses 5'→3' polymerase, 5'→3' exonuclease and 3'→5' exonuclease activities. It is the principal enzyme for DNA replication in *E. coli*. Its core enzyme has an MW of about 180 kdal.

Events in replication

A single DNA polymerase simultaneously replicates the growing strands on the unwound segments of both template strands in a replication bubble. But both the DNA template strands cannot be copied simultaneously and continuously in the same direction, because (i) the two template strands are antiparallel to each other, (ii) they unwind only for short stretches at a time, and (iii) DNA polymerases can copy each strand in a 3'→5' direction only. So, the polymerase

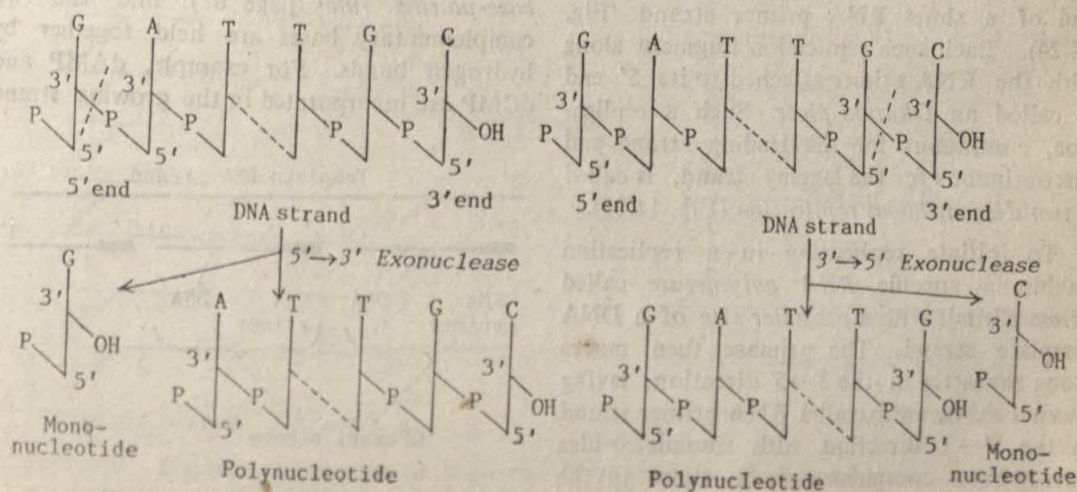


Fig. 14.23. 5'→3' exonuclease and 3'→5' exonuclease actions.

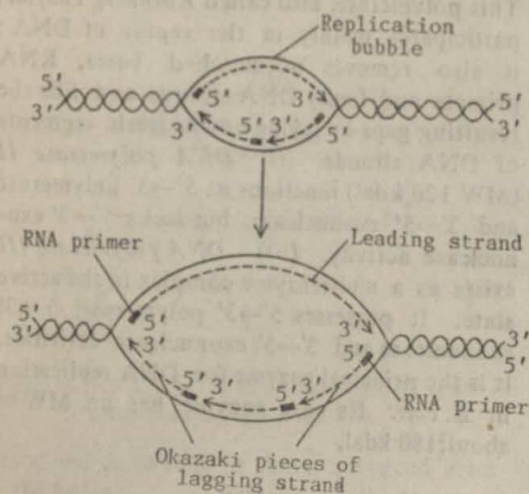


Fig. 14.24. Discontinuous laying down of the lagging strand as Okazaki pieces.

replicates one growing DNA strand (*leading strand*) continuously in the $5' \rightarrow 3'$ direction starting from the $3'$ end of a single RNA primer strand; but it replicates the other growing strand (*lagging strand*) discontinuously in the form of many separate short *replication fragments*, each made of about 150-200 deoxyribonucleotides and laid down in a $5' \rightarrow 3'$ direction starting from the $3'$ end of a short RNA primer strand (Fig. 14.24). Each such replication fragment along with the RNA primer attached to its $5'$ end is called an *Okazaki piece*. Such a replication, continuous for the leading strand and discontinuous for the lagging strand, is called a *semidiscontinuous replication* (Fig. 14.25).

To initiate replication in a replication bubble, a specific *RNA polymerase* called *primase* binds with a *promoter site* of a DNA template strand. The primase then moves along the latter in the $3' \rightarrow 5'$ direction, laying down a short, antiparallel RNA primer strand in the $5' \rightarrow 3'$ direction with ribonucleotides having bases complementary to those in the DNA template.

After the RNA primer has been laid down over the specific segment of the template strand, the base of the next deoxyribonucleotide of the latter gets hydrogen-bonded to a complementary base of a deoxyribonucleoside $5'$ -triphosphate from the surrounding medium. *DNA polymerase* then catalyzes a nucleophilic attack by the terminal $3'$ -OH of the RNA primer on the α -phosphate of the incoming deoxyribonucleoside $5'$ -triphosphate. This releases PPi from the latter and links the resulting deoxyribonucleoside $5'$ -phosphate with the $3'$ -OH of the RNA by a phosphodiester bond (Fig. 14.26). In the same way, *DNA polymerase* continues adding new deoxyribonucleotides successively at the $3'$ end of the growing DNA strand by forming a phosphodiester bond at each step between the $3'$ -OH of the last deoxyribonucleotide incorporated and the $5'$ -phosphate of the next deoxyribonucleotide coming from the corresponding deoxyribonucleoside $5'$ -triphosphate. The bases of the deoxyribonucleotides in the template strand determine the deoxyribonucleotides to be incorporated at the corresponding positions of the growing strand—each added nucleotide must bear a base complementary to that of the corresponding template deoxyribonucleotide as per the *Watson-Crick base-pairing rule* (page 81) and the two complementary bases are held together by hydrogen bonds. For example, dAMP and dCMP are incorporated in the growing strand

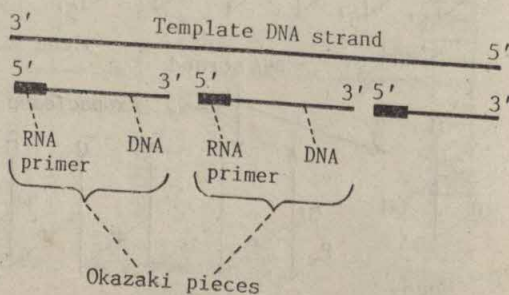


Fig. 14.25. Semidiscontinuous replication.

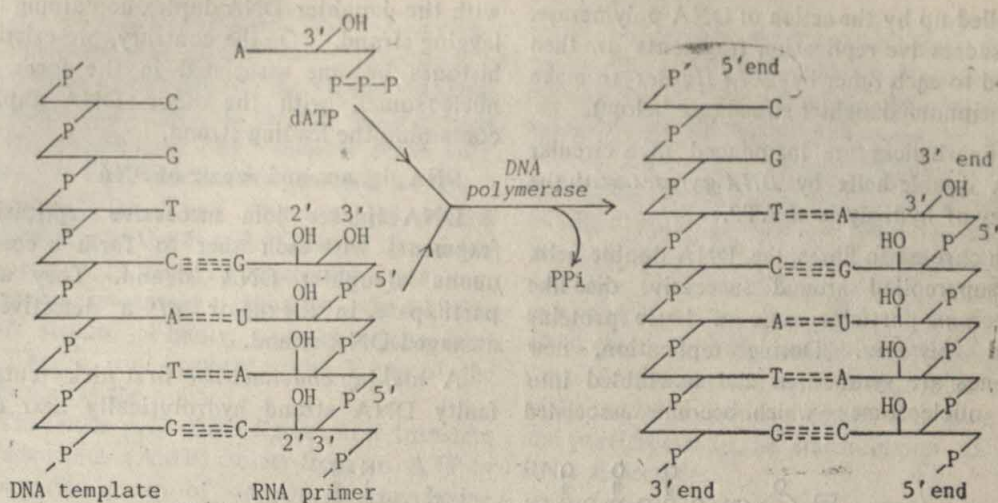


Fig. 14.26. Addition of a deoxyribonucleotide with a complementary base to the RNA primer.

against respectively dTMP and dGMP of the template strand and vice versa (Fig. 14.27). This makes the template strand and its daughter strand complementary to each other in base sequence and also ensures that the ratio $(A+T)/(C+G)$ is identical in the two. The two strands, antiparallel to each other, progressively wind together to form ultimately

a new DNA double-helix held by hydrogen bonds between the complementary bases of its two strands.

With the progress of replication, a 5'→3' exonuclease hydrolytically removes the 5'-terminal RNA primers from both the leading strand and the Okazaki pieces destined to form the lagging strand. The resulting gaps

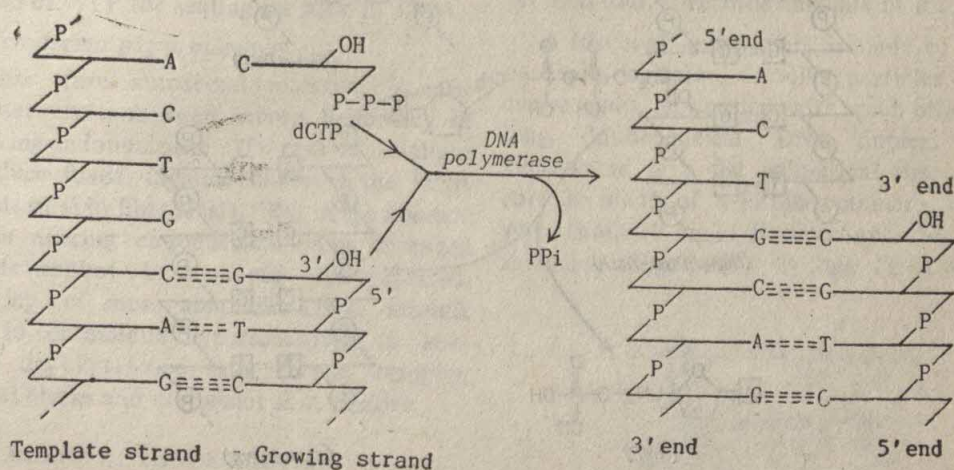


Fig. 14.27. Elongation of a growing DNA strand by incorporating a complementary deoxyribonucleotide at its 3' end.

are filled up by the action of DNA polymerase. The successive replication fragments are then joined to each other by *DNA ligases* to make a continuous daughter strand (see below).

Superhelices are introduced in a circular DNA double-helix by *DNA gyrases* with the energy of hydrolysis of ATP.

In chromatin fibres, the DNA double-helix lies supercoiled around successive disc-like *nucleosome* particles made of basic proteins called *histones*. During replication, new histones are synthesized and assembled into new nucleosomes which become associated

with the daughter DNA duplex containing the lagging strand. On the contrary, pre-existing histones become associated in the form of nucleosomes with the other DNA duplex containing the leading strand.

DNA ligases and repair of DNA

DNA ligases join successive replication fragments with each other to form a continuous daughter DNA strand. They also participate in the repair of a defective or damaged DNA strand.

A *nicking endonuclease* first nicks (cuts) a faulty DNA strand hydrolytically near one

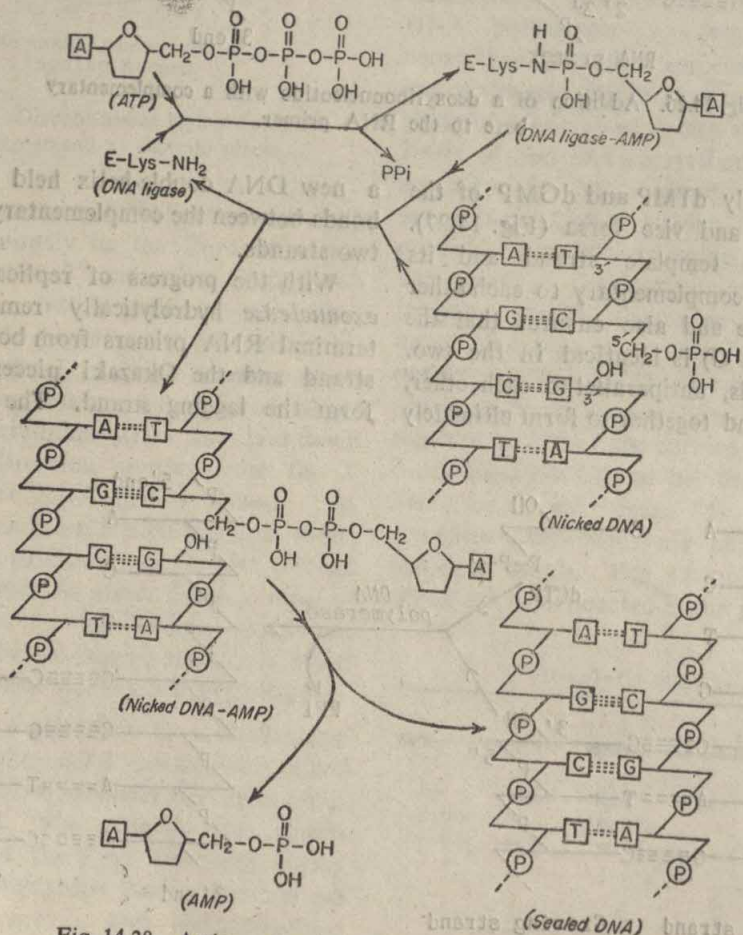


Fig. 14.28. Action of eukaryotic DNA ligase in sealing a nicked DNA strand.

end of its defective region. An *exonuclease* then sequentially removes the nucleotides of the defective segment of the strand, starting from its nicked end. In the gap thus created, a DNA polymerase (e.g., nuclear *DNA polymerase β*) lays down a new stretch of DNA strand in the 5'→3' direction by adding segments such as deoxyribonucleotides as bear bases complementary to those of the faultless DNA strand. Finally, a *DNA ligase* joins the 5' end of one segment to the 3' end of the other across the nick.

The *eukaryotic DNA ligase* first transfers the adenylate (AMP) moiety from an ATP to the ϵ -amino group of one of its own lysine residues, forming a ligase-adenylate complex and releasing PPi (Fig. 14.28). This complex transfers its adenylate moiety to the terminal 5'-phosphate of one of the DNA chain fragments, activating that phosphate group. The ligase next catalyzes a nucleophilic-attack on that activated phosphorus atom by the terminal 3'-OH of the other chain fragment. This forms a phosphodiester bond joining the 5' end of one fragment with the 3' end of the other and releasing the AMP.

The DNA ligase of *E. coli* uses NAD⁺ instead of ATP for sealing the nick in DNA.

Xeroderma pigmentosum :

This rare autosomal recessive genetic disorder results from an inborn deficiency of a nicking endonuclease. UV rays of sunlight introduce fused thymine dimers in the DNA strands of skin fibroblasts. But in the absence of the nicking endonuclease, such damaged strands cannot be removed and repaired. Thriving of these abnormal DNA strands leads to cutaneous hypersensitivity to sunlight, dry keratosed skin, dermal atrophy, corneal ulcers and malignant skin tumors.

14.5 REVERSE TRANSCRIPTION

Retroviruses or oncogenic RNA viruses have a *reverse transcriptase* which functions as

an *RNA-directed DNA polymerase*. Inside the host cell, it uses a viral RNA strand as a template to synthesize a complementary DNA strand so that the two strands form a DNA-RNA hybrid duplex. The enzyme *RNase H* then hydrolytically removes the RNA strand of this hybrid to leave a DNA transcript of the viral RNA. Then, the reverse transcriptase uses this DNA transcript as the template to synthesize a complementary DNA strand which winds with the template DNA strand to form a double-helical viral DNA. The latter acts as a *DNA provirus* inside the host cell and participates in the transcription of viral RNA molecules.

14.6 CHROMATIN

Chromatin is the nuclear chromosomal material. In the interphase nucleus of non-dividing cells, it may be seen as thin diffuse filaments and coarse granules scattered in the nucleoplasm and in small masses inside the inner nuclear membrane.

Chromatin is composed of *double-helical DNA* molecules closely associated with aggregates of small basic proteins called *histones*, smaller amounts of *nonhistone acidic proteins* and some little amounts of *RNA*.

A 10-nm chromatin fibril is made of a row of dense, circular, disc-like particles called *nucleosomes*, connected with each other by a long double-helical DNA duplex. Each nucleosome is a flat cylindrical disc whose core is made of a histone octamer. It measures about 10 nm in diameter and 5-6 nm in thickness (Fig. 14.29). A long DNA duplex

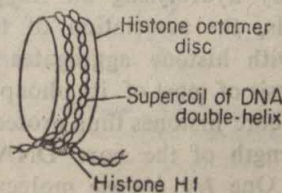


Fig. 14.29. A nucleosome.

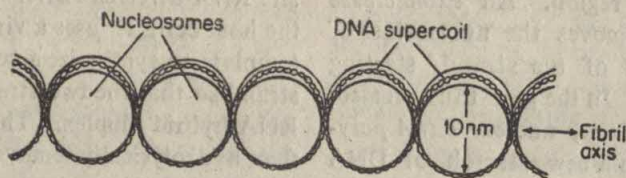


Fig. 14.30. A 10-nm chromatin fibril.

strand coils successively in a superhelix around the circular rim of many such nucleosomes, wrapping each with 1.75 left-handed turns with a length of about 140 base-pairs and a pitch of about 2.8 nm. This places the nucleosomes serially side by side with their circular rims in contact with each other and their flat faces parallel to the long axis of the 10-nm fibril (Fig. 14.30). The short stretch of the DNA double strand, which runs between and joins the successive nucleosomes, is called the *linker DNA* and makes the chromatin fibril flexible. The stretch of the DNA strand, which winds around the nucleosome, is called the *core DNA*. The histone octamer, forming the disc-like core of the nucleosome, is a tightly bound aggregate of one $H3_2$ - $H4_2$ tetramer made of two molecules each of arginine-rich H3 and H4 histones, and two $H2A$ - $H2B$ dimers of one molecule each of lysine-rich H2A and H2B histones. These core histones carry most of their basic amino acid residues in the N-terminal third of their peptide chains. The superhelix of the core DNA makes contact with the core histones in the order H2A-H2B-H4-H3-H3-H4-H2B-H2A, while winding around the nucleosome. While nucleases such as pancreatic DNase I can degrade free DNA completely by hydrolyzing all its phosphodiester bonds, the association of the DNA superhelix with histone aggregates prevents such hydrolysis of most of its phosphodiester bonds—the core histones thus protect a 146-base-pair length of the core DNA against hydrolysis. One *H1* histone molecule binds peripherally and rather loosely to each nucleo-

some near the point of entry and departure of the linker DNA, helping to seal the DNA double-helix to the nucleosome, protecting another 20-base-pair length of the DNA against hydrolysis, stabilizing further supercoils of the 10-nm fibril and thereby promoting the superpacking of the nucleosomes. The nucleosome is assembled in this way through specific interactions between histones and DNA, promoted by an anionic pentameric nucleoplasmic protein called *nucleoplasmin*.

A 30-nm chromatin fibre is constituted in the interphase and metaphase nuclei by the supercoiling of the 10-nm chromatin fibril. The latter gets coiled into a tight solenoidal superhelix with about 6 interconnected nucleosomes per turn (Fig. 14.31). During metaphase and anaphase, the 30-nm fibre gets coiled more than hundredfold into short, dense and thick fibres to constitute the *chromosome*. Each chromatid of the latter possesses a single double-helical DNA molecule, made on average of about 1.5×10^8 base-pairs in humans. Such higher orders of

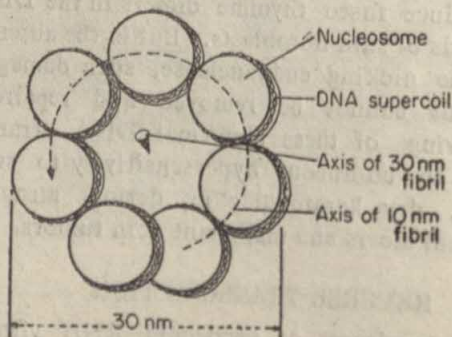


Fig. 14.31. Coil of a 30-nm chromatin fibre.

chromatin structure may need for stabilization some nonhistone proteins in addition to H1 histone. Chromatin fibres of metaphase chromosomes form many loops of even 10^5 base-pair length, which may surround and bind to a scaffold made of nonhistone proteins.

During the replication of DNA, the new DNA duplex containing the leading DNA strand retains the histones previously associated with the parent DNA duplex while the other new DNA duplex with the lagging strand gets new histones synthesized afresh.

Chromatin that exists as dense thick fibres and remains inactive without participating in RNA transcription even during interphase and early prophase, is called *heterochromatin*. Non-condensed, less dense chromatin which participates actively and regularly in RNA transcription, is called *euchromatin*. The heterochromatin of chromosomal telomeres and centromeres never uncoils to participate in transcription and is called *constitutive heterochromatin*. The heterochromatin of one X chromosome of mammalian females sometimes uncoils to participate in transcription and is called *facultative heterochromatin*.

14.7 TRANSCRIPTION OF RNA

An RNA molecule is primarily synthesized by the polymerization of ribonucleotides on the template of a particular region of a DNA strand, serving as one or more genes. The genetic information contained in the base sequence of the genes of DNA is transcribed thereby into the base sequence of the RNA. The RNA molecule, initially synthesized, is known as a *primary transcript*.

Different regions of one strand of the DNA double-helix serve as the *template strand*, *sense strand* or *coding strand* for transcribing different species of RNA molecules. The corresponding regions of the complementary DNA strand are *noncoding* and do not serve as the template for any RNA transcription

(*asymmetric transcription*); but other regions of even that strand function as the coding strand for other species of RNA. Thus, each strand of a DNA double-helix codes for several species of RNA although both strands do not code for the same species of RNA.

The RNA strand is transcribed as anti-parallel to its coding DNA strand. Its base sequence is complementary to that of the coding strand, but identical with that of the noncoding DNA strand except that the latter carries thymine residues corresponding to uracil residues of the transcribed RNA.

The two strands of the DNA double-helix partially unwind over about 17 base-pairs at a time to expose the bases of the coding strand for transcription. After the unwound region of the coding strand has been transcribed, the two strands rewind simultaneously with the unwinding of the next segment of the DNA duplex. Thus, on completion of transcription, the DNA double-helix regains its original structure through the rewinding of its original strands. This is called *conservative transcription* in contrast to the semiconservative replication (Fig. 14.32).

RNA polymerases

These enzymes lay down a primary RNA transcript on the template of the coding

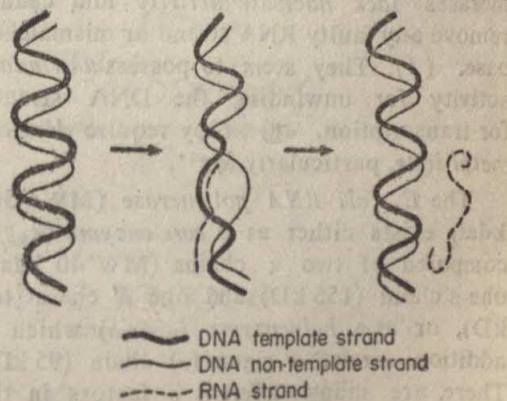


Fig. 14.32. Conservative transcription.

DNA strand. They show following characteristics. (a) Cellular RNA polymerases, like the DNA polymerases, require a *DNA template*. The base sequence of the latter determines the sequence in which ribonucleotides are incorporated by the RNA polymerase in the growing RNA strand. These RNA polymerases are thus called *DNA-dependent RNA polymerases*. (b) They resemble DNA polymerases in functioning as 5'→3' polymerases, i.e., they extend the growing RNA strand in the 5'→3' direction by catalyzing the formation of a phosphodiester bond between the 5'-phosphate of a new ribonucleotide and the 3'-OH of the 3'-terminal ribonucleotide of the already laid segment of the RNA strand. Because the growing RNA strand and its coding DNA strand are antiparallel, RNA polymerases copy the latter in a 3'→5' direction. (c) Unlike DNA polymerases, RNA polymerases require *no primer strand*; instead, they can directly initiate the RNA chain, incorporating either guanosine 5'-triphosphate (pppG) or adenosine 5'-triphosphate (pppA) as the 5'-terminal nucleotide of the chain. (d) RNA polymerases require the presence of *all four ribonucleoside 5'-triphosphates*, viz., ATP, GTP, CTP and UTP, which they utilize as substrates for incorporating new ribonucleotides in the RNA chain. (e) RNA polymerases *lack nuclease activity* and cannot remove any faulty RNA strand or mismatched base. (f) They seem to possess *unwindase* activity for unwinding the DNA strands for transcription. (g) They require *divalent metal ions*, particularly Mg^{2+} .

The *E. coli* RNA polymerase (MW ≈ 500 kdal) exists either as a *core enzyme* ($\alpha_2\beta\beta'$) composed of two α chains (MW 40 kdal), one β chain (155 kD) and one β' chain (165 kD), or as a *holoenzyme* ($\alpha_2\beta\beta'\sigma$) which in addition carries a sigma (σ) chain (95 kD). There are many different σ factors in the *E. coli* cell, each with a specificity for binding

with a *promoter site* at the 5' end of specific coding strands. According to the σ factor bound to the polymerase, the holoenzyme binds with the promoter of a specific coding strand, initiates its transcription and then releases the σ subunit to change into the core enzyme. The latter thereafter continues elongating the growing RNA transcript. The binding sites for ribonucleoside 5'-triphosphates, to be used as substrates, are located probably in the β subunit of the core enzyme. On completion of the transcription, the core polymerase is released from the coding strand and may bind with a different σ factor; the holopolymerase now binds with the promoter site of a different coding strand to initiate its transcription. The termination of transcription and the release of the core polymerase from the coding strand are signalled by the binding of a tetrameric *rho* (ρ) protein (MW 200 kdal) with a *termination site* at the 3' end of the coding strand.

Mammalian RNA polymerases belong to three major classes. All of them are oligomeric macromolecules (MW 500-600 kdal). Each is made of two larger subunits (>100 kdal) and several smaller subunits (<100 kdal). No protein acting as σ or ρ factor has been identified for them. (i) *Class I RNA polymerases* occur in nucleoli, require Mg^{2+} and low ionic strengths for optimum activity, synthesize primary RNA transcripts (45S) for rRNA, and are not inhibited by the mushroom poison α -amanitin. (ii) *Class II RNA polymerases* occur in the nucleoplasm, synthesize heterogeneous nuclear RNA (hnRNA) transcripts meant for mRNA, and are inhibited by very low concentrations (10^{-8} M) of amanitin. (iii) *Class III RNA polymerases* are also nucleoplasmic, synthesize primary RNA transcripts for low MW RNAs (tRNA and 5S RNA) and are affected by much higher concentrations (10^{-4} M) of amanitin. The nucleoplasmic polymerases require higher ionic strengths and

The binding of the holopolymerase with the DNA is immediately followed by the local unwinding of the double-helix (Fig. 14.33). The pliability of the Pribnow box, resulting from its lack of GC base-pairs,

facilitates the separation of the two DNA strands. This gives the polymerase an easy access to the nucleotides in the following stretch of the coding (sense) strand. After the unwinding, the base of the pyrimidine deoxy-

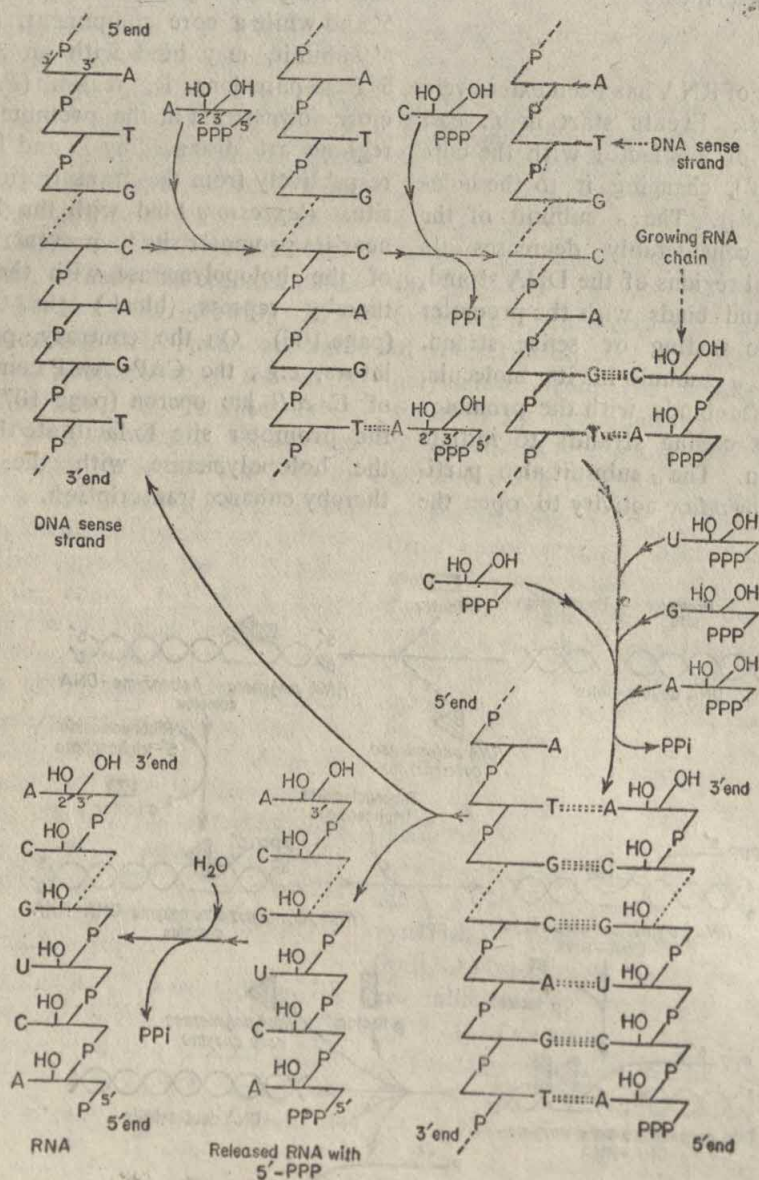


Fig. 14.34. Transcription in the 5'→3' direction utilizing ribonucleoside triphosphates and a DNA coding strand (sense strand).

ribonucleotide (dTMP or dCMP) at the transcription start site and that of the next deoxyribonucleotide in the coding strand get hydrogen-bonded to the complementary bases of respectively a purine ribonucleoside 5'-triphosphate (ATP or GTP) and a second purine or pyrimidine ribonucleoside triphosphate. The polymerase catalyzes a nucleophilic attack by the 3'-OH of the first purine ribonucleotide on the α -phosphate of the second ribonucleotide. This releases PPI from the latter and links its 5'-phosphate with the 3'-OH of the first ribonucleotide by a phosphodiester bond to form a dinucleoside tetraphosphate: $\text{pppA (or pppG)} + \text{pppX} \rightarrow \text{PPI} + \text{pppApX (or pppGpX)}$. This dinucleoside tetraphosphate has either pppA or pppG as the 5'-terminal ribonucleotide, a 5'-triphosphate group at its 5' end and a 3'-OH group at its 3' terminus. After the first internucleotide phosphodiester linkage has been formed between either ATP or GTP and the next ribonucleotide specified by the coding strand, the polymerase releases the σ factor and changes into the core enzyme ($\alpha_2\beta\beta'$). As the latter has less affinity for the promoter site, it can travel onwards along the coding strand to continue the transcription.

The antibiotics *rifampicin* and *streptovaricin* bind with the β subunit of the poly-

merase to block the initiation of transcription. Rifampicin probably competitively inhibits the enzyme by binding with its ATP/GTP-binding site.

Elongation:

As the core polymerase moves along the coding strand in the 3'→5' direction, it adds successive ribonucleotides at the 3'-OH terminus of the ribonucleotide chain already laid down, making the latter grow in the 5'→3' direction. At each step, the incoming ribonucleotide is donated by the corresponding ribonucleoside 5'-triphosphate and gets linked by a phosphodiester bond between its 5'-phosphate and the terminal 3'-OH of the preceding ribonucleotide. The bases of the coding DNA strand determine the ribonucleotides to be incorporated at the corresponding positions of the growing RNA strand—each added nucleotide must bear a base complementary to that of the corresponding template deoxyribonucleotide as per the *base-pairing rule* (page 81).

The antibiotic *actinomycin D* forms complexes with double-helical DNA by getting intercalated between two guanine-cytosine base-pairs in DNA, thereby prevents the movement of the core polymerase along the DNA and consequently inhibits the elongation stage

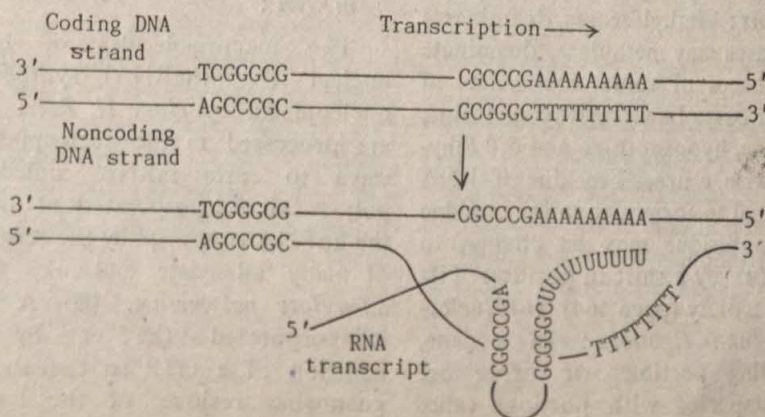


Fig. 14.35. Termination signal for bacterial transcription.

of transcription. *Streptolydigin* binds with the β subunit of *E. coli* polymerase and thereby inhibits the elongation stage.

Termination :

The DNA template carries a *transcription termination signal* in the form of a specific base sequence in the coding strand. In *E. coli*, this signal consists of two inverted GC-rich regions, separated by an intervening region and followed by an AT-rich region with a series of adenine residues in the coding strand (Fig. 14.35). The RNA transcript of this region has a poly-U tail preceded by a hair-pin loop due to hydrogen-bonding of complementary guanine and cytosine residues in its two stretches. These structural features and the binding of the rho (ρ) protein with the termination site of the DNA terminate the transcription and release the RNA transcript and the core polymerase from the DNA.

The initiation and termination signals for mammalian transcription are far more complex and involve interactions of specific DNA sequences with specific proteins.

14.8 POST-TRANSCRIPTIONAL PROCESSING OF RNA

The primary RNA transcript, released from polysomes, is differentiated into mRNA, rRNA and tRNA in eukaryotes. (a) *Nucleoside modifications* : Methyltransferases, deaminases and dehydrogenases may methylate, deaminate or reduce the bases of some nucleosides of RNA into minor bases like 5-methylcystosine, N⁶-methyladenine, hypoxanthine and 5,6-dihydrouracil. Likewise, a uracil residue of tRNA may be methylated to form a thymine residue while a uridine residue may be changed to pseudouridine (ψ) by a shift in position of its phosphoribosyl moiety (page 464). (b) *Nucleolytic cleavages and ligations* : In a gene, amino acid-coding portions or *exons* are frequently interspersed with portions called *introns* which do not finally contribute to the

amino acid sequence of the protein. But the RNA transcript carries the transcripts of introns between those of the exons of its gene. However, specific *nucleases* remove the ribonucleotides of intron transcripts while the exon transcripts, consequently separated from each other, are spliced together by *ligases*. These change the primary RNA transcript into a mature RNA before it may pass from the nucleus to the cytoplasm. (c) *Terminal addition* : Additional nucleotides may be added at either end of the RNA transcript. These include the addition of a poly-adenylate (poly-A) tail and a GTP cap (5-pppG) at respectively the 3' and 5' ends of an mRNA (page 82).

rRNA :

Nucleolar *endonucleases* cleave the 45S RNA transcript, synthesized by *Class I RNA polymerases* in nucleoli, into 5.8S, 18S and 28S rRNA molecules and several inactive *spacer* fragments. Change of some uridines to pseudouridines (page 464), the methylation of some bases and the 2'-O-methylation of some ribose residues are brought about in either the 45S RNA or its subsequent products. 5.8S and 28S rRNAs are combined with proteins to form 60S ribosomal subunits; 18S rRNA molecules combine with proteins to constitute 40S ribosomal subunits.

mRNA :

The macromolecules of heterogeneous nuclear RNA (hnRNA), synthesized in the nucleoplasm by *class II RNA polymerases*, are processed in the nucleoplasm in several ways to form mRNA molecules. (i) A poly-A tail is incorporated at the 3' end of the hnRNA transcript by the terminal addition of many adenylate molecules by a *poly-adenylate polymerase*. (ii) A 5'-pppG cap is incorporated at the 5' end by the terminal addition of a GTP so that the 5'-C of the guanosine residue of the latter and the terminal 5'-C of the RNA transcript are

bridged by a 5'-5' triphosphate bridge (Fig. 4.11). (iii) The N⁷ of the guanine residue of the 5'-pppG cap is methylated. (iv) Intron transcripts are then removed from the hnRNA by nucleolytic cleavage with the help of endonucleases. The exon transcripts, separated from each other by the cleavage of intervening introns, are spliced together by ligases to produce the mRNA molecule. (v) The 2'-OH of some of the nucleotides of mRNA are methylated.

tRNA :

Primary RNA transcripts, synthesized in the nucleoplasm by *Class III RNA polymerases*, are processed into tRNA molecules, partly in the nucleoplasm and partly in the cytoplasm. (i) Some of the bases such as guanine, cytosine and hypoxanthine are methylated in the nucleoplasm. (ii) One or more uracil residues, destined for the D loop of the tRNA being processed, are reduced to dihydrouracil. (iii) Another uracil residue, destined to lie in the T loop of the tRNA, is methylated to thymine. (iv) Some uridines are changed to pseudouridines by a shift of the phosphoribosyl moiety from N¹ to C⁵ of its pyrimidine ring (page 464). (v) Cytoplasmic endonucleases cleave off stretches of the RNA transcript to reduce its size or to divide it into more than one tRNA molecule. (vi) Endonucleases also cleave off intron transcripts near the anticodon loop of the tRNA and ligases splice the anticodon region properly. (vii) In the cytoplasm, *tRNA CMP-AMP pyrophosphorylases* utilize CTP and ATP to carry out the terminal addition of a cytidine-cytidine-adenosine (CCA) terminus at the 3' end of the tRNA molecule.

14.9 GENETIC CODE

Base sequences of DNA constitute genetic messages for determining hereditary characters. These coded messages are transcribed into complementary base sequences of mRNA.

A *genetic code* is constituted by the base sequence in a part of the mRNA molecule. This is translated into a specific sequence of amino acids in the peptide chain synthesized with the participation of the relevant part of the mRNA strand. The genetic code consists of many *codons*. Each codon is a triplet of bases in three consecutive ribonucleotides of the mRNA and directs the incorporation of a specific amino acid in the peptide. *Polycistronic mRNA* of prokaryotes carries more than one genetic code, separated by intervening sequences of noncoding ribonucleotides. Such mRNA codes for as many species of peptide chains as the number of its genetic codes. Eukaryotic mRNA is *monocistronic*. Each such mRNA carries a single genetic code and codes for a single peptide species.

Characteristics of genetic code :

(a) *Unambiguity*: Any specific codon codes for a single specific amino acid only and cannot incorporate any other amino acid in the peptide chain.

(b) *Universality*: Each particular amino acid is coded by the same codon or codons in all species of organisms. But such universality has following exceptions: (i) AUA, the codon for isoleucine in the cytoplasm, codes instead for methionine in mammalian mitochondria. (ii) UGA, not coding for any amino acid but serving to signal the termination of peptide synthesis in the cytoplasm, serves to code for tryptophan in mitochondria. (iii) AGA and AGG code for arginine in the cytoplasm, but signals the termination of the synthesis of a peptide in mammalian mitochondria instead of coding for any amino acid.

(c) *Degeneracy*: Except methionine and tryptophan, each amino acid is coded by more than one codon. Any of the latter may incorporate that amino acid in the peptide.

Valine, for example, is coded by GUU, GUC, GUA and GUG.

(d) *Nonoverlap*: Consecutive codons are distinct from and independent of each other, and do not overlap—the same base cannot function as a common member of two consecutive codons.

(e) *Commalessness*: There is no intervening nucleotide between two consecutive codons in a genetic code. The last nucleotide of one codon is immediately followed by the first nucleotide of the next codon.

Codons and their families:

Codons are read in the 5'→3' direction (shown as left→right) along the mRNA strand. Of the total of 64 codons, three are called *terminator* or *nonsense codons* because instead of incorporating any amino acid in the peptide, they signal the termination of translation. The remaining 61 codons code for 20 different amino acids (Table 14.1). One of the terminator codons, viz., UAA, UAG or UGA in the cytoplasm and AGA or AGG in mitochondria, is the last codon at the 3' end of the row of codons constituting the genetic code. The terminator codon is preceded on its 5' side by the codon coding for the C-terminal amino acid of the peptide. The translation of the latter always starts from a *chain initiator codon*, viz., AUG. This is the first codon at the 5' end of the genetic code and codes for the amino acid methionine at the N-terminal end of the peptide. AUG occurs at other sites of the genetic code also for incorporating methionine at the middle of the peptide chain.

Codons are often grouped into 16 *families*, each characterized by specific first two bases in all four of its members; e.g., the UU family consists of UUU, UUC, UUA and UUG while the GC family consists of GCU, GCC, GCA and GCG. A *mixed codon family* codes for more than one amino acid, depending on the third base in its codons;

TABLE 14.1. Genetic code (extramitochondrial).

Family	Codons	Amino acid
AA	AAA, AAG	Lys
	AAU, AAC	Asn
AC	ACA, ACC, ACG, ACU	Thr
AG	AGA, AGG	Arg
	AGC, AGU	Ser
AU	AUA, AUC, AUU	Ile
	AUG	Met
CA	CAA, CAG	Gln
	CAC, CAU	His
CC	CCA, CCC, CCG, CCU	Pro
CG	CGA, CGC, CGG, CGU	Arg
CU	CUA, CUC, CUG, CUU	Leu
GA	GAA, GAG	Glu
	GAC, GAU	Asp
GC	GCA, GCC, GCG, GCU	Ala
GG	GGA, GGC, GGG, GGU	Gly
GU	GUA, GUC, GUG, GUU	Val
UA	UAA, UAG	Terminator
	UAC, UAU	Tyr
UC	UCA, UCC, UCG, UCU	Ser
UG	UGA	Terminator
	UGC, UGU	Cys
UU	UGG	Trp
	UUA, UUG	Leu
	UUC, UUU	Phe

e.g., in the UU family, UUU and UUC code for phenylalanine but UUA and UUG code for leucine. On the contrary, all four codons of an *unmixed codon family* code for the same amino acid irrespective of the third base; e.g., in the GC family, GCU, GCA, GCC and GCG all code for alanine.

First two bases of a codon are more specific in coding the amino acid; a change in any of these two bases changes the family of the codon and also in most cases, the coded amino acid. The third base of the codon is less specific for coding and its replacement by some other base does not change the coded amino acid in many cases,

e.g., in the unmixed families. This is responsible for the *degeneracy* of the code (see above).

14.10 TRANSLATION OF PROTEINS

A protein or peptide is synthesized by the polymerization of amino acids, with peptide linkages joining the α -amino and α -carboxyl groups of successive amino acids. Besides enzymes and the proteins acting as initiation, elongation and release factors, ribosomes and a specific mRNA molecule are involved in the process. Amino acids are incorporated in the peptide chain, starting from its N-terminal end and following a specific sequence

determined by the sequence of codons in the genetic code of the relevant mRNA. In other words, the genetic message carried by the genetic code is *translated* into the amino acid sequence of the protein.

Translation takes place in the cytoplasm in prokaryotes. In eukaryotes, cytoplasmic proteins are translated in the cytosol, secretory proteins and most membrane proteins are synthesized on the rough endoplasmic reticulum while some of the mitochondrial proteins are synthesized in mitochondria.

Formation of amino acyl-tRNA

In the cytoplasm, each *amino acyl-tRNA synthetase* possesses specificities for a parti-

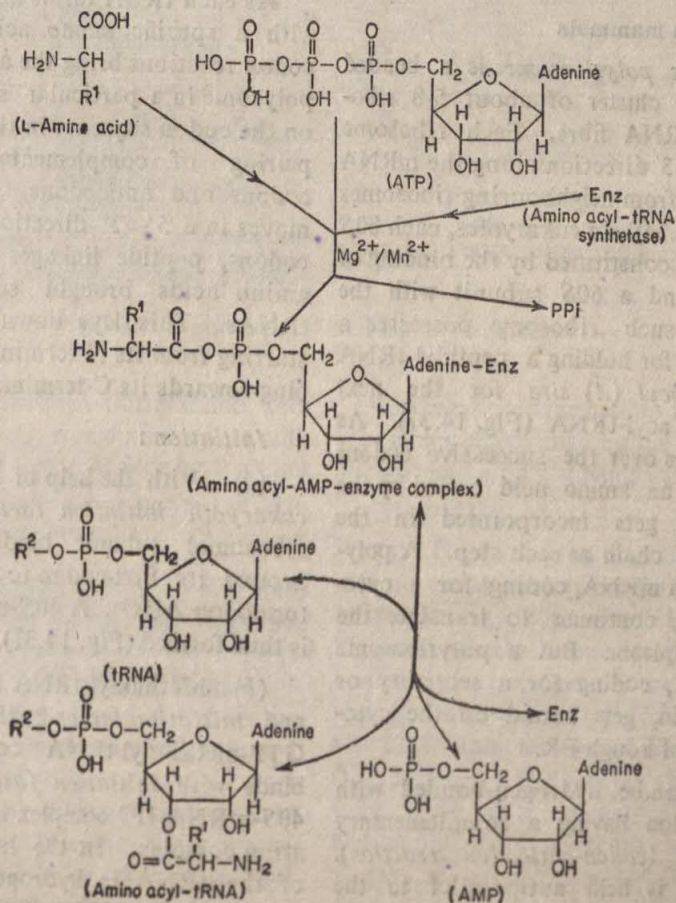


Fig. 14.36. Formation of amino acyl-tRNA.

cular L-amino acid and also for a specific tRNA. The interaction between the specific L-amino acid, ATP and the specific amino acyl-tRNA synthetase in presence of Mg^{2+} or Mn^{2+} produces PPi and an activated amino acyl-AMP-enzyme complex (Fig. 14.36). The latter then transfers the amino acid to a specific tRNA which is recognized by the enzyme at least partly by its D loop (page 84). This transfer results in forming an amino acyl-tRNA complex and in releasing the free enzyme and AMP. In the amino acyl-tRNA, the α -carboxyl group of the amino acid remains esterified with the 3'-OH of the 3'-terminal adenosine on the acceptor arm of tRNA.

Translation in mammals

A *polysome* or *polyribosome* is a beaded string-like linear cluster of about 5-8 ribosomes on an mRNA fibre. Each ribosome moves in the 5'→3' direction along the mRNA strand, separated from neighbouring ribosomes by 5-7 nm (Fig. 4.14). In eukaryotes, each 80S cytoribosome is constituted by the binding of a 40S subunit and a 60S subunit with the mRNA. Each such ribosome possesses a *peptidyl (P) site* for holding a peptidyl-tRNA and an *amino acyl (A) site* for the next incoming amino acyl-tRNA (Fig. 14.37). As a ribosome moves over the successive codons of the mRNA, an amino acid coded by the relevant codon gets incorporated in the growing peptide chain at each step. A polyribosome with an mRNA, coding for a cytoplasmic protein, continues to translate the latter in the cytoplasm. But a polyribosome with an mRNA, coding for a secretory or membrane protein, gets bound on the cytoplasmic surface of rough ER.

Each codon can be hydrogen-bonded with a tRNA anticodon having a complementary triplet of bases (*codon-anticodon reaction*). As the tRNA is held antiparallel to the mRNA, the bases 1, 2 and 3 (counted 5'→3')

of the anticodon can pair respectively with the complementary bases 3, 2 and 1 of the codon, obeying the *base-pairing rule*. According to *Crick's wobble hypothesis*, the first base of an anticodon may sometimes pair with different third bases of codons, provided the third and second bases of the anticodon can form the standard complementary pairs with respectively the first and second bases of the codon. Such wobble permits a pairing of, say, the first base uracil of an anticodon with the third base guanine of a codon, and makes the third base of a codon less specific for coding an amino acid. So, the same amino acid may sometimes be coded by more than one codon differing in their third bases.

As each tRNA forms an amino acyl-tRNA with a specific amino acid, the codon-anticodon reactions bring the amino acids to the polysome in a particular sequence depending on the codon sequence in the mRNA and the pairing of complementary bases between codons and anticodons. As each ribosome moves in a 5'→3' direction over consecutive codons, peptide linkages are formed between amino acids brought sequentially by the tRNAs. This lays down the peptide chain, starting from its N-terminal end and advancing towards its C-terminal end.

Initiation :

(a) With the help of a protein called the *eukaryotic initiation factor 3 (IF3)*, a 40S ribosomal subunit binds with an mRNA around its first codon to be translated (initiator codon AUG). A 40S-mRNA-IF3 complex is thus formed (Fig. 14.37).

(b) Methionyl-tRNA combines with GTP and *initiation factor 2 (IF2)* to form an IF2-GTP-methionyl-tRNA complex. The latter binds with *initiation factor 1 (IF1)* and the 40S-mRNA-IF3 complex to give a 40S initiation complex. In the latter, the anticodon of the tRNA is hydrogen-bonded with the initiator codon AUG of the mRNA.

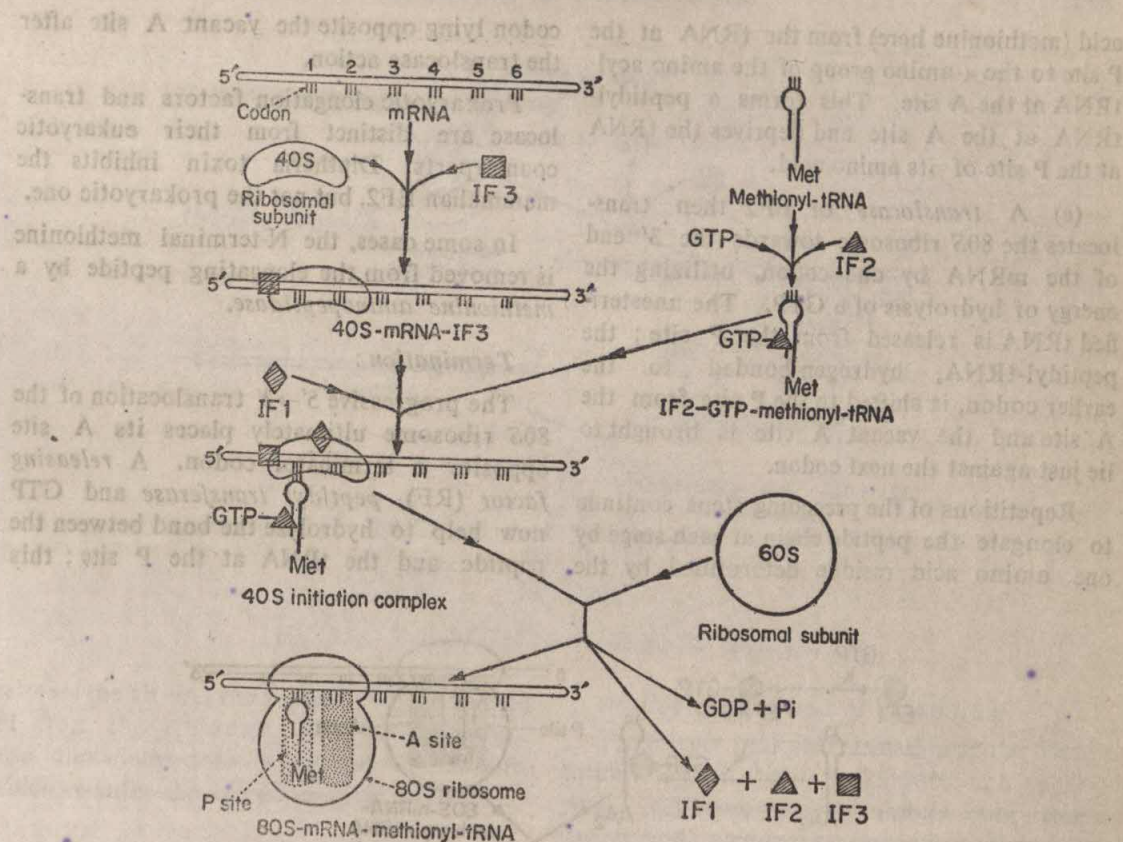


Fig. 14.37. Initiation of translation in mammals.

Unlike eukaryotic translation starting with methionine, prokaryotic translation starts with N-formylmethionine as the N-terminal amino acid. Prokaryotic initiation factors are also distinct from eukaryotic ones.

(c) A 60S ribosomal subunit next binds with the 40S subunit of the initiation complex, constituting an 80S ribosome on the mRNA. The GTP held by the initiation complex is simultaneously hydrolyzed and GDP, Pi and the three initiation factors are all released. The 80S ribosome lies with its A site opposite the codon next to the initiator codon AUG while its P site lies opposite the initiator codon and is occupied by the methionyl-tRNA, hydrogen-bonded to that codon.

Prokaryotic translation is done by 70S ribosomes constituted by the binding of a 30S subunit and a 50S subunit with mRNA.

Elongation :

(a) Depending on the codon lying opposite to the A site, a specific amino acyl-tRNA next gets hydrogen-bonded to that codon. This involves the formation of an intermediate complex of an *elongation factor* (EF1), GTP and the new amino acyl-tRNA, hydrolysis of the GTP and release of EF1-GDP and Pi (Fig. 14.38). EF1-GDP is changed to EF1-GTP again with the help of protein factors and another GTP molecule.

(b) A *peptidyl transferase* of the 60S ribosomal subunit next transfers the amino

acid (methionine here) from the tRNA at the P site to the α -amino group of the amino acyl-tRNA at the A site. This forms a peptidyl-tRNA at the A site and deprives the tRNA at the P site of its amino acid.

(c) A *translocase* or EF2 then translocates the 80S ribosome towards the 3' end of the mRNA by one codon, utilizing the energy of hydrolysis of a GTP. The unesterified tRNA is released from the P site; the peptidyl-tRNA, hydrogen-bonded to the earlier codon, is shifted to the P site from the A site and the vacant A site is brought to lie just against the next codon.

Repetitions of the preceding steps continue to elongate the peptide chain at each stage by one amino acid residue determined by the

codon lying opposite the vacant A site after the translocase action.

Prokaryotic elongation factors and translocase are distinct from their eukaryotic counterparts. Diphtheria toxin inhibits the mammalian EF2, but not the prokaryotic one.

In some cases, the N-terminal methionine is removed from the elongating peptide by a *methionine aminopeptidase*.

Termination :

The progressive 5'→3' translocation of the 80S ribosome ultimately places its A site opposite a terminator codon. A *releasing factor* (RF), *peptidyl transferase* and GTP now help to hydrolyze the bond between the peptide and the tRNA at the P site; this

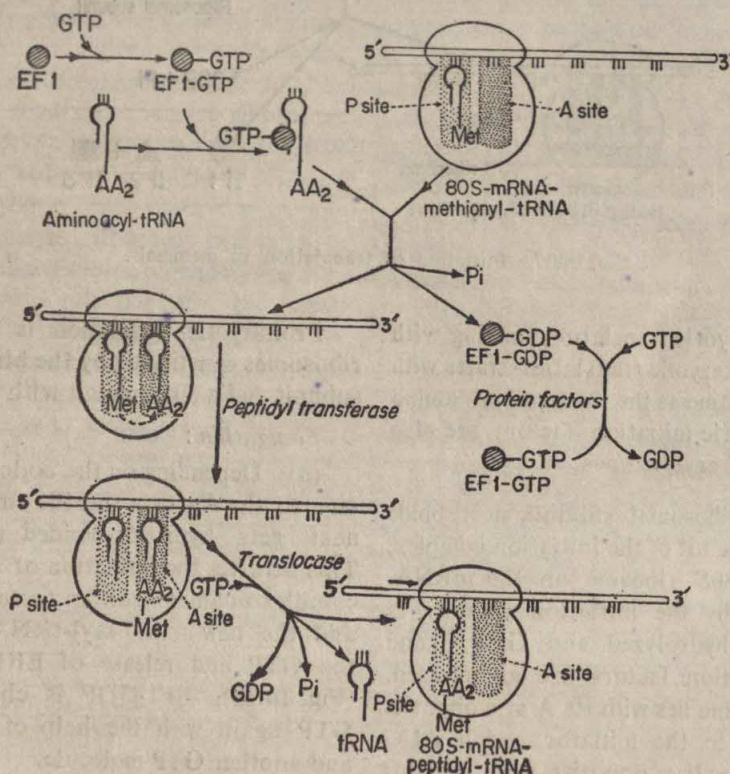


Fig. 14 38. Elongation of peptide chain in mammals.
AA₂ : second amino acid.

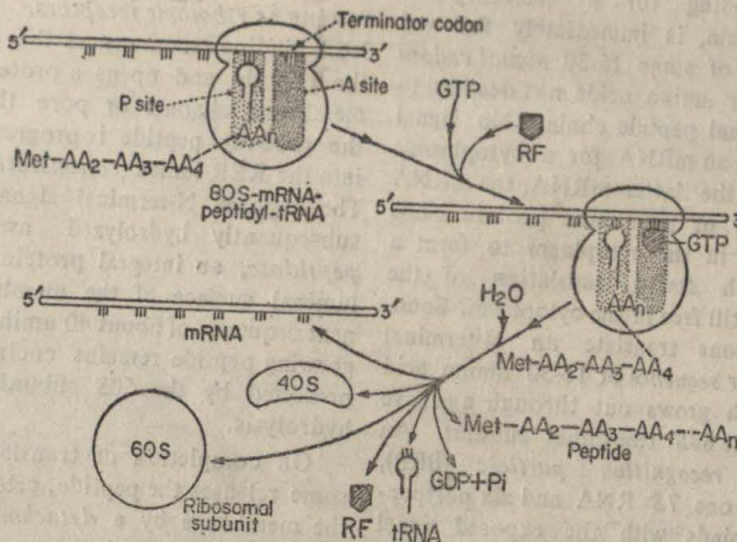


Fig. 14.39. Termination of translation in mammals.

releases the tRNA, the peptide, RF, GDP and Pi from the ribosome. The 80S ribosome also dissociates into its 60S and 40S subunits which re-enter the cytoplasm (Fig. 14.39).

Energy expenditure :

Incorporation of each amino acid in the peptide involves the expenditure of 4 high-energy phosphate bonds. (a) During the formation of amino acyl-tRNA, 2 high-energy bonds are spent due the hydrolysis of ATP to AMP and PPi. (b) Incorporation of the amino acyl-tRNA at the A site causes the loss of one high-energy bond due to the hydrolysis of GTP to GDP and Pi. (c) Another high-energy bond of GTP is spent during translocase action.

At the termination of translation, one more high-energy bond of GTP is spent for the release of the peptide.

Thus, in synthesizing a peptide with n number of amino acids, the energy expenditure amounts to high-energy bonds numbering $4n + 1$.

Antibiotic inhibitors of translation :

Puromycin inhibits translation at the elongation stage in both prokaryotes and eukaryotes. *Chloramphenicol* inhibits both elongation and termination stages in prokaryotes and eukaryotic mitochondria, but not in eukaryotic cytoplasm. *Tetracycline* inhibits the elongation stage in prokaryotes and eukaryotic mitochondria while *erythromycin* inhibits the termination stage in both; but neither of these antibiotics affect translation in eukaryotic cytoplasm.

14.11 SYNTHESIS OF MEMBRANE PROTEINS

Secretory proteins, membrane proteins and lysosomal enzymes are translated by polyosomes attached to the cytoplasmic surface of rough ER membrane. There are two hypotheses about the translation of membrane proteins and their incorporation into membranes.

Signal hypothesis

According to this hypothesis of Blobel and Sabatini (1971), the chain initiator codon of

an mRNA, coding for a secretory or membrane protein, is immediately followed by a sequence of some 15-30 *signal codons* which code for amino acids not destined to remain in the final peptide chain. No signal codon occurs in an mRNA for a cytoplasmic protein. Like the latter mRNA, the mRNA for a secretory or membrane protein binds with ribosomes in the cytoplasm to form a polysome which starts translation of the protein while still free in the cytoplasm. Soon, the signal codons translate an N-terminal *signal or leader sequence* of 15-30 amino acid residues, which grows out through a groove or tunnel in the 60S ribosomal subunit. An 11S *signal recognition particle* (SRP), composed of one 7S RNA and six polypeptides, then binds with the exposed signal sequence and arrests further translation by the time the peptide has lengthened to about 70 amino acid residues (Fig. 14.40).

Translation is resumed only after the ribosome binds on the cytoplasmic surface of the rough ER (RER) membrane in three ways: (a) initially by hydrophobic interactions between nonpolar groups of the membrane and nonpolar amino acid side-chains of the signal sequence, (b) SRP-mediated linking of the 60S subunit with an integral membrane protein called the *SRP receptor* or *docking protein*, and (c) ionic bonds between the 60S ribosomal subunit and

integral membrane proteins called *ribophorins* serving as *ribosome receptors*. The ribosome-RER binding recommences the translation of the peptide and opens a protein-lined trans-membrane channel or pore through which the growing peptide is progressively inserted into the RER lumen (*cotranslational insertion*). The exposed N-terminal signal sequence is subsequently hydrolyzed away by *signal peptidase*, an integral protein opening on the luminal surface of the membrane; but the next sequence of about 40 amino acids in the growing peptide remains encircled and thus, protected by the 60S subunit against such hydrolysis.

On completion of translation, the ribosome releases the peptide, gets separated from the membrane by a *detachment factor*, and re-enters the cytoplasm as 40S and 60S subunits. The peptide, if destined to be a secretory protein, is released free into the RER lumen and proceeds thence to the lumen of Golgi cisternae. But peptides, destined to be integral membrane proteins, continue to span the thickness of the RER membrane even after their release from the ribosome, because their C-terminal region has either a short hydrophobic segment embedded and hydrophobically bound into the membrane lipid bilayer, or a short hydrophilic segment anchored to the polar groups on the membrane surface. Thus, such proteins are not

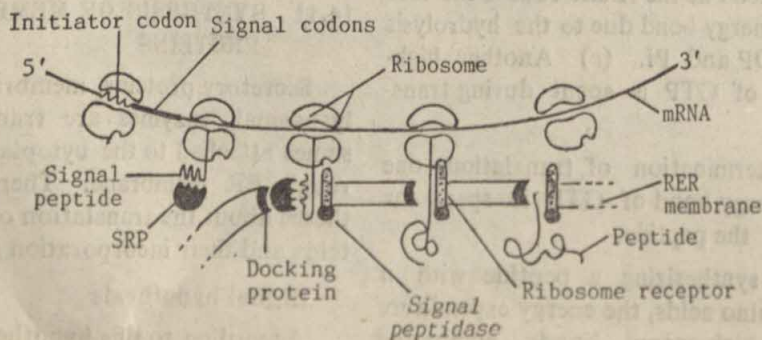


Fig. 14.40. Signal hypothesis.

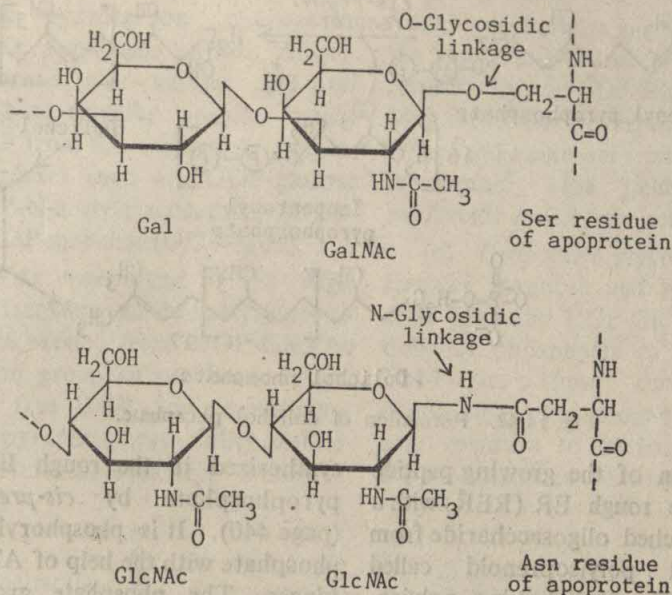


Fig. 14.41. Glycosidic linkages in O-linked and N-linked glycoproteins.

released free into the RER lumen; instead they move towards the smooth ER and Golgi cisternae by lateral diffusion along the membrane.

Membrane trigger hypothesis

According to this hypothesis, a cytoplasmic polysome, unattached to the RER membrane, synthesizes hydrophobic membrane peptides. Each of the latter bears a N-terminal *leader sequence* which endows it with three-dimensional coils hiding its hydrophobic domains in the interior of the coils and consequently keeping it soluble in the aqueous cytosol. During or after the translation, the peptide gets associated with either the membrane lipid bilayer or a membrane receptor protein. This triggers a conformational change in the peptide, probably exposing some of its hydrophobic domains and thereby enabling it to be inserted and oriented in the lipid bilayer. No specific ribosome-membrane interaction seems necessary for these changes. The leader sequence of the peptide is then

hydrolyzed away and the resulting protein assumes its intended intramembrane conformation, depending largely on its own primary structure.

The trigger mechanism may coexist with the signal mechanism in the cell.

14.12 GLYCOSYLATION OF PROTEINS

Glycosylation of secretory and membrane proteins is started in the rough ER (RER) even before the peptide chain is released from its ribosome. However, the glycosylation continues in the smooth ER (SER) and is completed in the Golgi complex.

Synthesis of N-linked glycoproteins

Majority of membrane-bound and circulating glycoproteins, including most serum globulins, belong to this class. In these, an N-glycosidic linkage joins the sidechain amide-N of an asparagine residue of the apoprotein with the C¹ of the terminal N-acetylglucosamine (GlcNAc) residue of a branched oligosaccharide (Fig. 14.41).

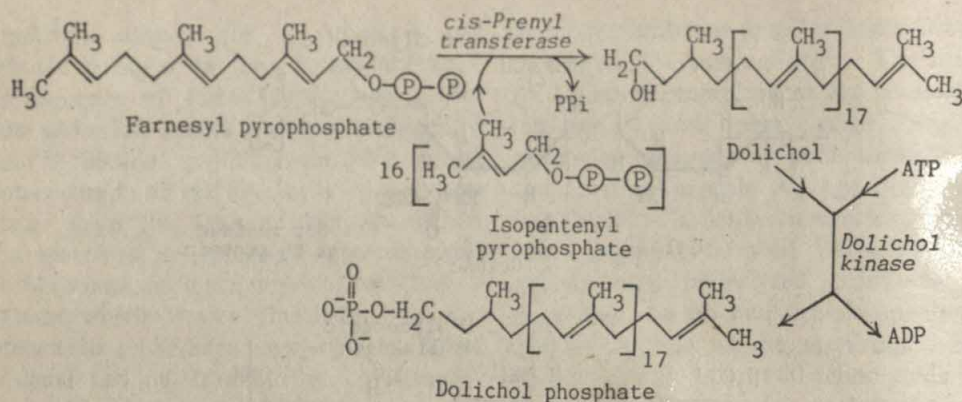


Fig. 14.42. Formation of dolichol phosphate.

The glycosylation of the growing peptide chain starts in the rough ER (RER) with a transfer of a branched oligosaccharide from a phosphorylated polyisoprenoid called *dolichol* (Fig. 14.42). Dolichol is a polyisoprenoid of 17-20 isoprenoid units and is

synthesized in the rough ER from farnesyl pyrophosphate by *cis-prenyl transferase* (page 440). It is phosphorylated to dolichol phosphate with the help of ATP and *dolichol kinase*. The phosphate group of dolichol phosphate (Dol-P) remains exposed on the

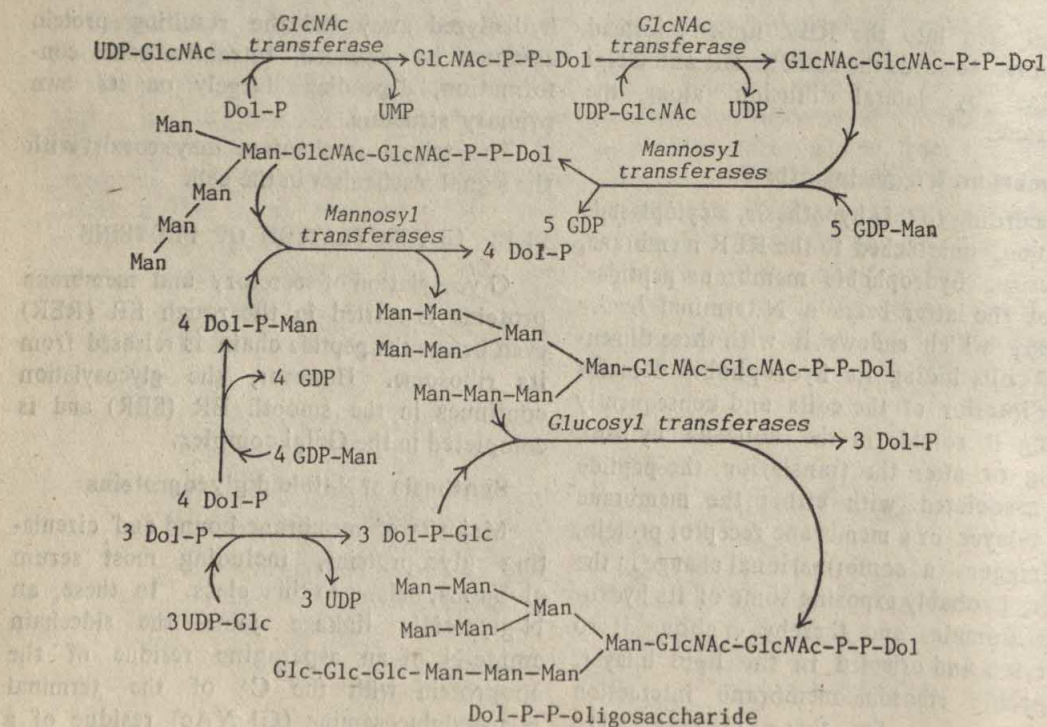


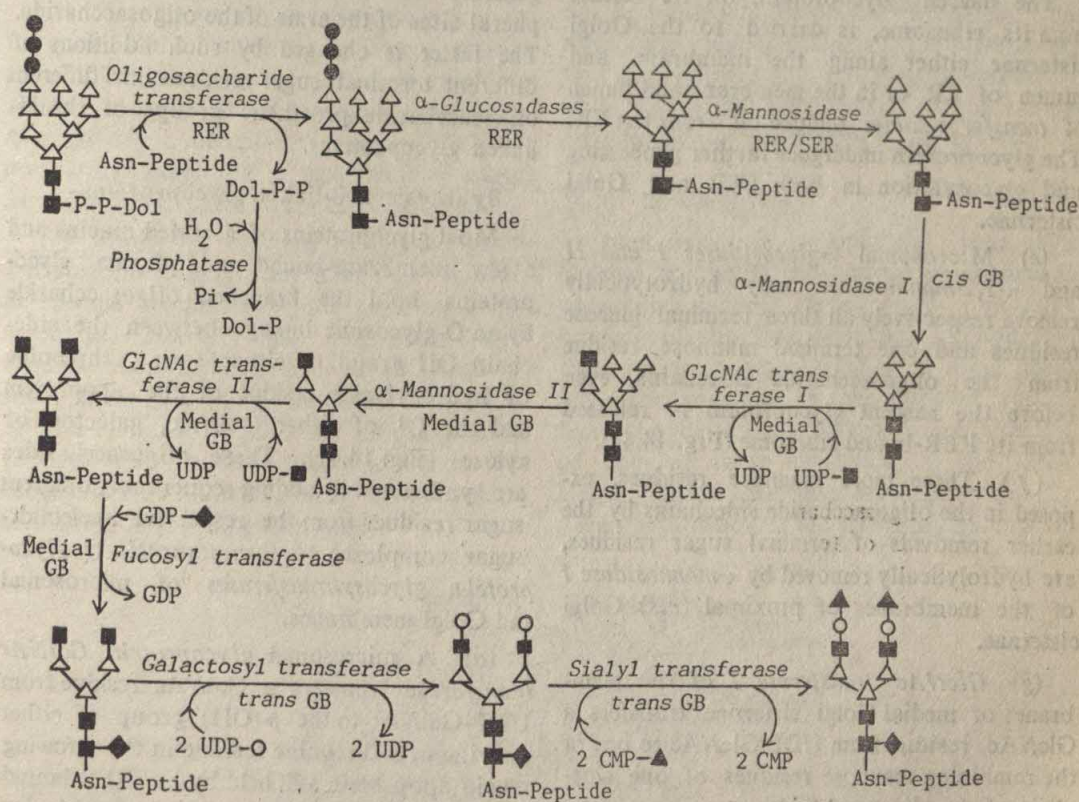
Fig. 14.43. Synthesis of Dol-P-P-oligosaccharide.

luminal surface of the RER membrane while its 10 nm-long hydrocarbon chain lies embedded in the membrane lipid bilayer. The ER membrane also carries *glycosyl transferases*. These transfer specific sugar residues to Dol-P from the respective nucleotide-sugar complexes such as UDP-glucose (UDP-Glc), UDP-N-acetylglucosamine (UDP-GlcNAc) and GDP-mannose (GDP-Man).

(a) A *GlcNAc transferase* of the RER membrane first transfers an N-acetylglucosamine 1-phosphate residue from UDP-GlcNAc to the phosphate group of membrane-bound Dol-P to yield free UMP and membrane-bound GlcNAc pyrophosphoryl-dolichol (GlcNAc-P-P-Dol) primer molecule (Fig. 14.43).

(b) Another GlcNAc and five mannose (Man) residues are successively transferred to the GlcNAc-P-P-Dol primer molecule from respectively one UDP-GlcNAc and five GDP-Man molecules by respectively *GlcNAc transferase* and *mannosyl transferases* of the RER membrane. This yields a nascent oligosaccharide moiety projecting in the ER lumen.

(c) Respective glycosyl transferases then transfer mannose and glucose residues from GDP-Man and UDP-Glc to membrane-bound dolichol phosphates to form Dol-P-Man and Dol-P-Glc. These successively serve as donors of four mannose residues and three glucose residues to the nascent oligosaccharide-P-P-Dol complex to form the high-mannose



● Glucose; Δ Mannose; ■ GlcNAc; ◆ Fucose; ○ Galactose; ▲ Sialic acid;
Dol-P: dolichol phosphate; Asn: asparagine; GB: Golgi body.

Fig. 14.44. Processing of the oligosaccharide prosthetic group of glycoproteins.

core oligosaccharide bound to Dol-P-P, viz., $(\text{Glc})_3(\text{Man})_9(\text{GlcNAc})_2\text{-P-P-Dol}$.

(d) A microsomal oligosaccharide transferase of RER transfers the core oligosaccharide en bloc to a specific asparagine residue of the growing peptide still held by its ribosome (Fig. 14.44). The oligosaccharide gets bound to the peptide by an N-glycosidic linkage between the C¹ of its terminal GlcNAc and the amide-N of Asn of the peptide, changing the latter to a nascent glycoprotein. The membrane-bound Dol-P-P, left behind by the oligosaccharide, is hydrolyzed by a phosphatase into Pi and Dol-P which participates in the next sequence of glycosylation.

The nascent glycoprotein, on its release from its ribosome, is carried to the Golgi cisternae either along the membrane and lumen of ER or in the membrane and lumen of transfer vesicles budded off from the ER. The glycoprotein undergoes further processing and glycosylation in both SER and Golgi cisternae.

(e) Microsomal α -glucosidases I and II and α -1,2-mannosidase may hydrolytically remove respectively all three terminal glucose residues and one terminal mannose residue from the oligosaccharide sidechains, even before the nascent glycoprotein is released from its RER-bound ribosome (Fig. 14.44).

(f) Three more mannose residues, exposed in the oligosaccharide sidechains by the earlier removals of terminal sugar residues, are hydrolytically removed by α -mannosidase I of the membranes of proximal (cis) Golgi cisternae.

(g) GlcNAc transferase I of the membranes of medial Golgi cisternae transfers a GlcNAc residue from UDP-GlcNAc to one of the remaining mannose residues of one sidechain of the oligosaccharide.

(h) α -Mannosidase II of the medial Golgi cisterna membranes then hydrolytically removes two mannose residues from another

sidechain of the oligosaccharide which thus becomes a biantennary structure with its first and second arms ending in GlcNAc and mannose residues respectively.

(i) In the medial Golgi cisternae, GlcNAc transferase II transfers a GlcNAc residue from UDP-GlcNAc to the terminal mannose of the second arm of the oligosaccharide.

(j) As the glycoprotein advances towards the distal cisternae near the concave face of the Golgi complex, fucosyl transferase, galactosyl transferase and sialyl transferase of the membrane of distal (trans) Golgi cisternae transfer L-fucose, D-galactose and sialic acid residues from respectively GDP-fucose, UDP-galactose and CMP-sialic acid to the peripheral sites of the arms of the oligosaccharide. The latter is changed by such additions of different terminal sugar residues into different oligosaccharide prosthetic groups of the N-linked glycoproteins.

Synthesis of O-linked glycoproteins

Most glycoproteins of secreted mucins and a few membrane-bound and plasma glycoproteins hold the branched oligosaccharide by an O-glycosidic linkage between the sidechain OH group of either serine, threonine or hydroxylysine residue of the apoprotein and the C¹ of either GalNAc, galactose or xylose (Fig. 14.41). These oligosaccharides are synthesized by adding sequentially different sugar residues from the respective nucleotide-sugar complexes to the apoprotein by glycoprotein glycosyltransferases of microsomal and Golgi membranes.

(a) A microsomal glycoprotein GalNAc transferase transfers a GalNAc residue from UDP-GalNAc to the β -(OH) group of either a serine or a threonine residue in the growing mucin apoprotein still held by its RER-bound ribosome (Fig. 14.45). A hydroxylysine residue of a growing collagen chain may similarly receive a galactose from UDP-Gal by the action of a microsomal glycoprotein galacto-

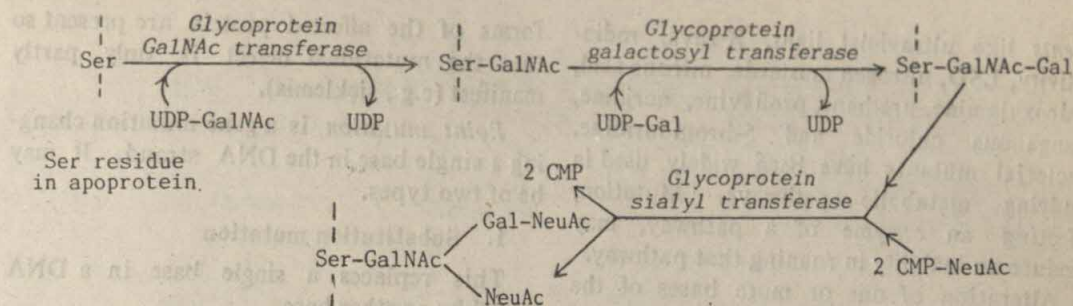


Fig. 14.45. Initial steps in the synthesis of O-linked glycoproteins.

syl transferase while a microsomal glycoprotein *xylosyl transferase* may add a xylose residue to a serine residue of a growing proteoglycan.

(b) A microsomal glycoprotein *galactosyl transferase* then transfers a galactose residue from UDP-galactose to the first GalNAc residue already added to the apoprotein. Alternatively, a microsomal glycoprotein *sialyl transferase* may add a sialic acid (NeuAc) from CMP-sialic acid to the GalNAc residue.

(c) Similarly, different membrane glycosyltransferases may add other sugar residues in specific sequences to the growing oligosaccharide chain. While the inner sugar residues such as GalNAc and galactose are added in the ER during or after translation of the peptide chain, the terminal sugar residues such as sialic acid and fucose are added to the branching arms of the oligosaccharide in the Golgi cisternae.

Both N-linked and O-linked glycoproteins are finally included in the *secretory vesicles* budded off from distal (*trans*) Golgi cisternae (Fig. 14.46). These vesicles marginate in the cell and fuse with its plasma membrane. The membrane glycoproteins of the vesicle are thus incorporated into the plasma membrane while the luminal glycoproteins of the vesicle are released outside the cell by exocytosis.

14.13 MUTATIONS

Mutations are abrupt and inheritable changes in the DNA, leading to changes in phenotypes. A *chromosomal mutation* consists of the loss, translocation, addition, exchange or positional rotation of some genes in the chromosome. A *gene mutation* results from the omission, addition or replacement of one or more nucleotides in the DNA strand. Mutations either arise spontaneously during replication or are caused by *mutagenic*

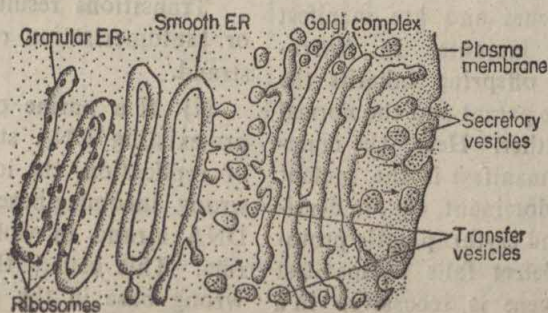


Fig. 14.46. Transport of materials from endoplasmic reticulum and Golgi complex.

agents like ultraviolet light, X-rays, radioactivity, LSD, nitrogen mustards, nitrous acid, hydroxylamine, urethane, proflavine, acridine, manganous chloride and 5-bromouridine. Bacterial mutants have been widely used in studying metabolic pathways. Mutation, affecting an enzyme of a pathway, may produce an inability in running that pathway.

Alteration of one or more bases of the DNA template strand by gene mutation leads to corresponding changes in the bases of one or more codons in the mRNA transcribed with the help of the mutant DNA. Ordinarily, mutation affects only one nucleotide of a codon. This results in the synthesis of an abnormal peptide differing from the normal peptide by one or more amino acids, each coded by an affected codon. Replication of the mutant DNA transmits the mutational change to the daughter cells arising from the mutant cell; all or half of the daughter cells carry the mutant DNA according as they are somatic or germ cells, respectively. As only half the germ cells of a parent contain the mutant gene, sometimes the offspring may not inherit any mutant gene. If both parents carry the mutant gene, the offspring may inherit the mutant gene from both parents to become a *homozygotic mutant*; in such a case, the biochemical defects due to the translation of the abnormal protein become manifest irrespective of the dominant or recessive nature of the mutant gene. Phenylketonuria and many other inborn biochemical defects are due to recessive mutant genes and are manifest only in homozygotic mutants. In *heterozygotic mutants*, the offspring inherits the mutant gene from one parent and the normal (wild) gene from the other. Here, the mutational defect is clearly manifest if the mutant gene is a Mendelian dominant, e.g., in dentinogenesis imperfecta and erythropoietic protoporphyria; but the defect fails to manifest itself if the mutant gene is recessive. In a heterozygotic mutant with a codominant mutant gene, both normal (wild) and mutant

forms of the affected protein are present so that the mutational defect is only partly manifest (e.g., sickle cell anemia).

Point mutation is a gene mutation changing a single base in the DNA strand. It may be of two types.

1. Substitution mutation

This replaces a single base in a DNA strand by another base.

(a) Transition:

Here, either a purine of the affected DNA strand is substituted by another purine, or a pyrimidine replaces another pyrimidine. During the subsequent replication, the corresponding base in the newly laid down complementary DNA strand is also substituted by a base complementary to the substituted base in the mutant strand. Thus, substitution of guanine by adenine in the mutant strand is followed by the substitution of the adjacent cytosine by thymine in the new complementary strand. Transition thus consists of a substitution of a guanine-cytosine base-pair by an adenine-thymine base-pair or vice versa, in the DNA double-helix. In other words, it replaces either a purine-pyrimidine base-pair by another purine-pyrimidine base-pair (AT $\rightleftharpoons$ GC) or a pyrimidine-purine base-pair by another pyrimidine-purine base-pair (TA $\rightleftharpoons$ CG). Transitions arise either spontaneously or due to mutagens like 2-amino-purine, 5-bromouridine or nitrous acid.

Transitions result from either ionization or tautomerization of the bases in a DNA strand.

(i) If a purine or pyrimidine base of the pre-existing DNA strand ionizes at the time of replication, the ionized base pairs with a wrong incoming base of the growing daughter DNA strand in violation of the base-pairing rule. This causes the incorporation of a wrong base in the growing strand at that point. Thus, ionized thymine of the pre-existing DNA strand pairs with guanine

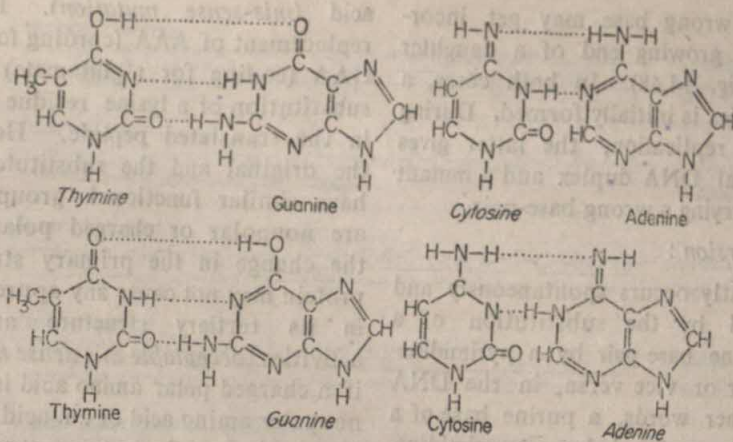


Fig. 14.47. Unusual base-pairing of rare tautomers (names in italics). Hydrogen bonds shown in dotted lines.

instead of adenine, and guanine is consequently incorporated in the growing DNA strand against the ionized thymine of the DNA coding (template) strand. This produces a *DNA heteroduplex*. During the next replication, the latter gives rise to a normal (wild) type of DNA duplex and a mutant DNA duplex having a new pyrimidine-purine base-pair in place of the old pyrimidine-purine base-pair (TA→CG).

(ii) Rare tautomeric forms of adenine, guanine, thymine and cytosine pair with cytosine, thymine, guanine and adenine,

respectively, instead of forming the conventional adenine-thymine or guanine-cytosine base-pairs (Fig. 14.47). Watson and Crick have suggested that base-pair transitions may frequently result from the tautomerization of a purine or pyrimidine base to its relatively rare form at the time of replication. This may happen in two ways. First, a base of the pre-existing coding DNA strand may change into its rare tautomer, causing a wrong complementary base to be incorporated against it in the growing DNA strand; secondly, a nucleotide carrying a rare

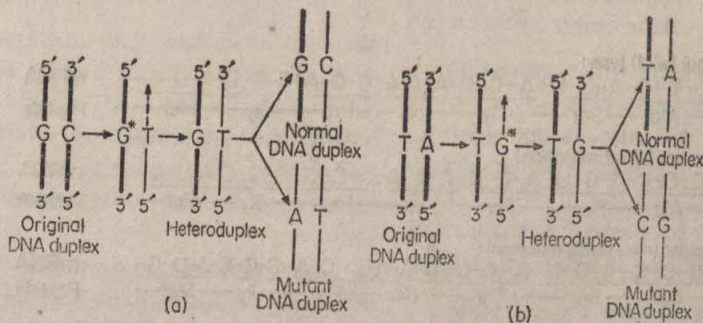


Fig. 14.48. (a) Transition due to tautomerism of a pre-existing base of DNA template. (b) Transition due to incorporation of the rare tautomer of a wrong base in the growing DNA strand. Rare tautomers marked with asterisks.

tautomer of a wrong base may get incorporated at the growing end of a daughter DNA strand (Fig. 14.48). In both cases, a DNA heteroduplex is initially formed. During the subsequent replication, the latter gives rise to a normal DNA duplex and a mutant DNA duplex carrying a wrong base-pair.

(b) *Transversion* :

This frequently occurs spontaneously and is characterized by the substitution of a purine-pyrimidine base-pair by a pyrimidine-purine base-pair or vice versa, in the DNA duplex. In other words, a purine base of a DNA strand is replaced by a pyrimidine, followed by the replacement of the corresponding pyrimidine base of the complementary strand by a purine, complementary to the newly substituted pyrimidine of the first strand.

Substitutions sometimes lead to a change of a codon to an alternative codon for an identical amino acid, e.g., change of CCA to CCG, both coding for L-proline (Fig. 14.49). In such cases, the corresponding peptide suffers no change in its amino acid sequence (*silent mutation*). About 75% of substitution mutations changes a codon to another, coding for a new amino acid. This makes the translated peptide differ from the normal peptide in respect of a single amino

acid (*mis-sense mutation*). For example, replacement of AAA (coding for L-lysine) by GAA (coding for L-glutamate) leads to the substitution of a lysine residue by glutamate in the translated peptide. However, where the original and the substituted amino acids have similar functional groups (e.g., both are nonpolar or charged polar amino acids), the change in the primary structure of the protein may not cause any appreciable change in its tertiary structure and biological activities (*acceptable mis-sense mutation*). But if a charged polar amino acid is replaced by a nonpolar amino acid or an acidic amino acid by a basic one, the tertiary structure may be so seriously affected that the biological activity of the protein may be partially or wholly lost (*partially acceptable and unacceptable mis-sense mutations*). Less than 5% of substitutions change a codon to a nonsense or terminator codon, leading to the formation of an inactive peptide shorter than the normal one by several C-terminal amino acids (*stop mutation*).

2. *Insertion or addition*

A point mutation may add an extra nucleotide in a DNA strand, leading to an insertion of an extra base-pair in the DNA duplex. Acridine and proflavine produce insertions. An insertion in the DNA coding strand results

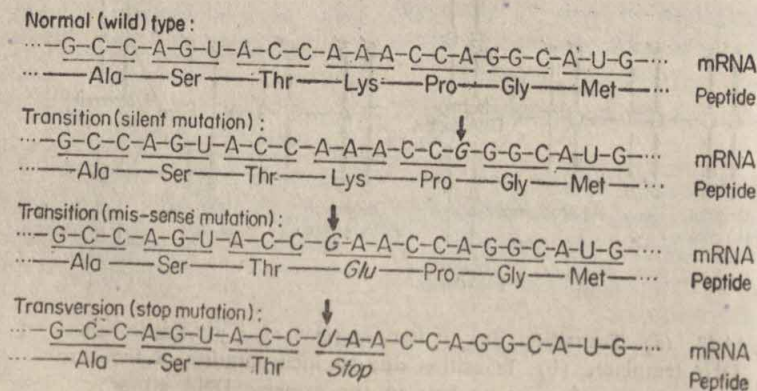


Fig. 14.49. Effects of transition and transversion on peptide synthesis.

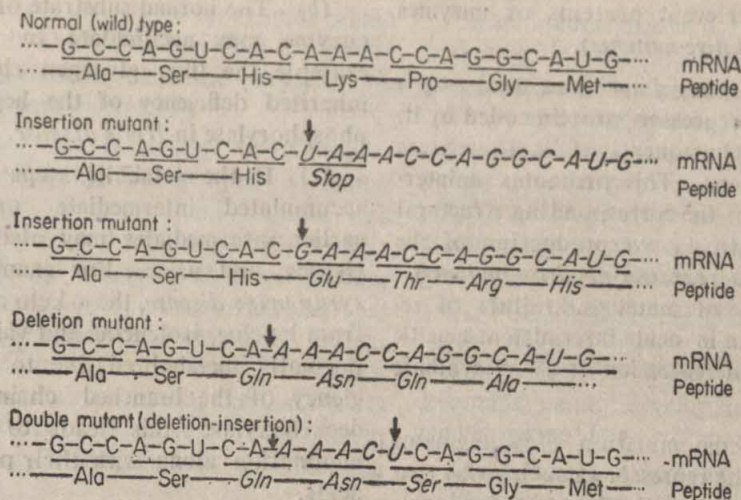


Fig. 14.50. Effects of frame-shift mutations on peptide synthesis.

in an addition of an extra base to the corresponding mRNA and consequently changes the base composition of all codons starting from the mutated point. Thus, from the point of mutation, each base triplet constitutes an abnormal codon and the affected mRNA translates either an abnormally shorter peptide or a grossly abnormal peptide with a large number of wrong amino acids (Fig. 14.50). Such mutations, frequently lethal, constitute one type of *frame-shift mutation*.

3. Deletion

A point mutation may omit a nucleotide from the DNA strand. A base-pair is consequently lost from the DNA duplex. This type of frame-shift mutation may be produced by proflavine, deaminating or alkylating agents, high temperature and very alkaline pH.

Besides point mutations, another class of mutations involves insertions and/or deletions of many base-pairs. Such mutations are also frame-shift mutations.

Sometimes, one insertion and one deletion may occur at a short distance from each other

on the same DNA so that two opposite frame-shifts annul each other and the original base sequence of each codon is reset after the second point of mutation (*suppression mutation*). In such cases, the translated peptide carries abnormal amino acids over only that part of the chain which is translated by the defective codons (Fig 14.50). Similarly, there is no frame-shift beyond the area of mutation if the mutation involves three insertions or three deletions; but in all such cases, the protein is defective over the mutant area due to the addition, omission or replacement of one or more amino acids.

Mutation of a structural gene leads to an altered amino acid composition of the peptide. In sickle cell anemia, for example, a mutant structural gene causes the synthesis of hemoglobin S with L-valine as the 6th amino acid in its β chain instead of L-glutamate at that position of normal hemoglobin A.

Mutation of an operator gene may prevent the binding of the repressor with the operator gene, allowing the latter to promote uninterrupted transcription by the corresponding structural genes with consequent overpro-

duction of the relevant proteins or enzymes (*operator-constitutive mutants*).

Mutation of a regulator gene leads to a change in the repressor protein coded by it. The repressor consequently fails to repress the operator gene. This promotes uninterrupted activity of the corresponding structural genes, leading to an overproduction of the relevant proteins (*repressor-negative mutants*).

Another type of mutational failure of repression is seen in acute intermittent hepatic porphyria with derepression of ALA synthase (page 265).

Sometimes, the mutation of a regulator gene changes the repressor in such a way that it continues to bind with the operator gene, but cannot bind any more with the inducer and consequently cannot be inactivated; thus, the operator gene continues to remain repressed and cannot be induced or derepressed (*super-repressed mutants*).

Sometimes, mutations may affect a DNA coding strand for tRNA synthesis. This results in the transcription of abnormal tRNA molecules and stops or alters translation of proteins, particularly when the mutation changes an anticodon of the tRNA.

14.14 INBORN ERRORS OF METABOLISM

Inborn errors of metabolism are inherited life-long diseases with mutational abnormalities of proteins. Most of them result from the loss of activity of a single specific enzyme and block the metabolic pathway requiring that enzyme.

(a) The loss of activity of an enzyme produces a deficiency of not only the final product of the relevant pathway but also the intermediates following the step catalyzed by that enzyme. Examples include the lack of melanin in *albinism* due to a genetic deficiency of tyrosinase, and low hepatic glycogen due to an inborn deficiency of the hepatic glycogen synthase in *glycogenosis IX*.

(b) The normal substrate of the defective enzyme may accumulate in tissues. For example, the liver glycogen rises due to an inherited deficiency of the hepatic glycogen phosphorylase in *Her's disease*.

(c) If the metabolic steps preceding the accumulated intermediate are reversible, earlier intermediates may also increase in tissues and urine. For example, in *maple syrup urine disease*, the α -keto acids produced from leucine, isoleucine and valine cannot be normally metabolized due to a genetic deficiency of the branched chain α -keto acid decarboxylase; this leads to their urinary elimination along with their precursor amino acids.

(d) A minor alternative pathway of the blocked intermediate may be manifold enhanced causing the accumulation and excretion of many abnormal products. In *phenylketonuria*, for example, a genetic deficiency of phenylalanine hydroxylase blocks the normal metabolism of phenylalanine; this enhances an alternative pathway, resulting in the overproduction and urinary elimination of phenylpyruvate, phenylacetate and phenyllactate.

(e) In operator-constitutive and repressor-negative mutations, a genetic failure of the repressor mechanism leads to an overproduction of the relevant enzyme, a consequent accumulation of the endproducts and by-products of the relevant pathway, and a simultaneous fall in other products arising from the substrate of the overactive enzyme through alternative pathways.

Mutation of a structural gene introduces structural abnormalities in the relevant enzyme, either reducing its stability or changing its substrate-binding or allosteric site. Mutation of an operator or regulator gene may reduce the amount of an enzyme without altering its structure. In both types of mutation, the end-result is a failure of activity of the affected enzyme. The inborn error may

also enhance the activity of an enzyme whose corepressor is the normal product of action of another genetically deficient enzyme (e.g., *acute intermittent porphyria*).

Some inborn errors produce gross abnormalities in growth, development, structure and functions of tissues and organs, and may often prove fatal; e.g., *phenylketonuria* characterized by severe mental retardation, and *maple syrup urine disease* characterized by mental retardation, respiratory failure, convulsive seizures and abnormal limb movements. Symptoms of some inborn errors like *alkaptonuria* and *albinism* are much milder and the patient may lead an otherwise healthy life.

Most inborn errors are manifest only in homozygotic mutants, because the mutant gene is often a Mendelian recessive gene (e.g., *albinism* and *phenylketonuria*). A few inborn errors like *dentinogenesis imperfecta*, *erythropoietic protoporphyria* and *acute intermittent porphyria* are manifest even in heterozygous mutants due to the dominant nature of the mutant gene. If the mutant gene is codominant, the homozygote develops a full manifestation of the inborn error (e.g., *sickle cell anemia*) while the defect, although clinically detectable, cannot develop fully in the heterozygotic mutant (e.g., *sicklelemlia*).

Many inborn errors have been described in different chapters of this book.

PHYSIOLOGICAL FUEL VALUE

The energy value of a food is the energy produced by the combustion of its protein, carbohydrate and fat. It is usually expressed in either kilocalories (kcal) or kilojoules (kJ). One kilocalorie is 1000 calories and one kilojoule is 1000 joules. The energy value of a food is usually expressed in kcal/g or kJ/g.

The energy value of a food is calculated by the heat liberated by its complete combustion in a calorimeter and is expressed in kcal/g or kJ/g. A measured mass of the food is placed in a bomb calorimeter and the heat liberated is measured. The food is completely oxidized by passing electric current through platinum wires embedded in the food. The results are corrected for heat capacity of the calorimeter and the energy released.

Combustion of 1 g of carbohydrate, protein or fat in the bomb calorimeter produces about 4.1 kcal and 17 kJ respectively. These are the actual energy values of the nutrients. But in the body, the food is not completely oxidized and the energy value is less. The energy value of a food in the body is called its physiological fuel value.

(5) Unlike carbohydrates and fats, proteins do not undergo complete combustion in the body to produce energy. A part of the absorbed protein is eliminated in the urine as NPN (nitrogenous products) whose energy value is measured in the bomb calorimeter, average 1.55 kcal/g or 6.5 kJ/g of protein metabolized.

Thus, the available energy from each gram of food protein, considering its DC of 9.25 and the unmetabolized NPN of 1.55 kcal, comes to: $(2.65 \times 0.92) + 1.55 = 4 \text{ kcal}$. Similarly, each gram of carbohydrate, in view of its DC of 9.25, yields in the body: $4.1 \times 0.92 = 3.8 \text{ kcal}$; each gram of food fat with a DC of 9.25 should yield in the body: $9.45 \times 0.92 = 8.7 \text{ kcal}$. These are the physiological fuel values of the respective nutrients and represent the energy actually available by their metabolism. The caloric value of a food is therefore, computed ordinarily by multiplying the amounts of carbohydrate, fat and protein in 100 g of the food by their respective physiological fuel values. For example, 100 g of whole wheat flour, containing 68 g carbohydrate, 12 g protein and 3 g fat, has a caloric value of $(68 \times 3.8) + (12 \times 8.7) + (3 \times 8.7) = 311 \text{ kcal}$.

15. HUMAN NUTRITION

Carbohydrates are required in the food mainly as fuels for energy production. Food proteins are principally needed for forming tissue proteins. Lipids of food serve as reserve fuels for energy and also as structural constituents. Besides these macronutrients, micronutrients such as vitamins and minerals are required in the diet for their diverse metabolic roles.

15.1 CALORIE VALUE AND PHYSIOLOGICAL FUEL VALUE

The *calorie value* of a food is the energy produced by the combustion of its proteins, carbohydrates and fats. It is usually expressed in either kilocalories ($1 \text{ kcal} = 10^3 \text{ cal}$) or megajoules (MJ); one MJ is 10^6 times the energy spent in moving 1 kg over 1 m by a force of 1 newton. ($1 \text{ kcal} = 4.184 \times 10^{-3} \text{ MJ}$).

The calorie value of a food is estimated by the heat, liberated by its complete combustion in a calorimeter and transferred to a known mass of water. A measured mass of the food is taken in a closed and O_2 -filled metal chamber (*bomb calorimeter*) placed in a known mass of water. The food is completely burnt by passing electric current through platinum wires embedded in the food. The resulting rise in water temperature is recorded for calculating the energy released.

Combustion of 1 g of carbohydrate, protein or fat in the bomb calorimeter produces about 4.1, 5.65 and 9.45 kcal respectively; these are the actual calorie values of the respective nutrients. But due to two reasons, the food liberates less energy in the body than its calorie value :

(a) A part of each food remains unabsorbed and is eliminated in the feces; the absorbed fraction is given by the *digestibility coefficient* (DC).

$$\text{DC} = \frac{100 \times (\text{food intake} - \text{fecal loss of unabsorbed food})}{\text{food intake}}$$

Generally, about 98% of dietary carbohydrates, 92% of proteins and 95% of fats are absorbed from food.

(b) Unlike carbohydrates and fats, proteins do not undergo complete combustion in the body to produce energy. A part of the absorbed protein is eliminated in the urine as NPN wasteproducts whose calorie values, measured in the bomb calorimeter, average 1.25 kcal per g of protein metabolized.

Thus, the available energy from each gram of food protein, considering its DC of 92% and the uncombusted NPN of 1.25 kcal, comes to : $(5.65 \times 0.92) - 1.25 \approx 4 \text{ kcal}$. Similarly, each gram of carbohydrate, in view of its DC of 98%, yields in the body : $4.1 \times 0.98 \approx 4 \text{ kcal}$; each gram of food fat with a DC of 95% should yield in the body : $9.45 \times 0.95 \approx 9 \text{ kcal}$. These are the *physiological fuel values* of the respective nutrients and represent the energy actually available by their metabolism. The calorie value of a food is, therefore, computed ordinarily by multiplying the amounts of carbohydrates, fats and proteins in 100 g of the food by their respective physiological fuel values. For example, 100 g of whole wheat flour, containing 69.6 g carbohydrate, 12 g protein and 1.5 g fat, has a calorie value : $4(69.6 + 12) + 9 \times 1.5 \approx 340 \text{ kcal}$.

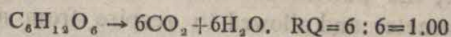
15.2 RESPIRATORY QUOTIENT

Respiratory quotient or RQ is the ratio of CO_2 produced and O_2 consumed by an individual in a given interval. It is also the ratio of the moles of CO_2 evolved and O_2 consumed in a given interval.

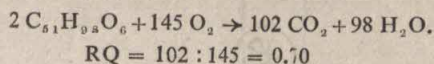
$$\text{RQ} = \frac{\text{volume of } \text{CO}_2 \text{ produced}}{\text{volume of } \text{O}_2 \text{ consumed}}$$

It is an indicator of the metabolic status.

(a) When carbohydrate alone is oxidized in the body, the moles of CO_2 produced equal in number the moles of O_2 consumed. So, RQ becomes 1.00.



(b) A fatty acid contains far less oxygen than a carbohydrate with identical number of carbons. So, when fat alone is oxidized, the moles of O_2 consumed from the inspiratory air, far exceed those of CO_2 produced. So, the RQ falls to nearly 0.7. For example, for tripalmitin ($\text{C}_{51}\text{H}_{98}\text{O}_6$),



(c) During lipogenesis from carbohydrates, the excess O_2 liberated from the latter is utilized for oxidations, reducing the amount of O_2 consumed from the inspired air. But because the CO_2 output is not lowered, it exceeds the amount of O_2 consumed, raising the RQ above 1.00.

(d) The RQ for protein oxidation is about 0.8.

(e) *Non-protein RQ* is the ratio of CO_2 produced and O_2 consumed for oxidizing food carbohydrates and fats, eliminating the effects of protein oxidation. Approximately 16 g of NPN are produced by the catabolism of 100 g proteins; so, the total amount of catabolized protein is obtained by multiplying the amount of urinary NPN with 100/16 or 6.25. The

CO_2 output and the O_2 consumption for this amount of protein are computed by multiplying the urinary NPN by 4.75 and 5.92 respectively, because each g of NPN represents the consumption of 5.92 litres of O_2 and the output of 4.75 litres of CO_2 for protein catabolism. The computed CO_2 output and O_2 consumption are deducted respectively from the total volumes of CO_2 output and O_2 consumption of the individual to get the volumes of CO_2 produced and O_2 consumed in oxidizing carbohydrates and fats alone. The ratio of the latter volumes of gases gives the non-protein RQ which depends on the proportionate amounts of carbohydrates and fats oxidized—the higher the proportion of fat oxidized, the closer is the RQ to 0.7. On an ordinary mixed diet, the non-protein RQ averages about 0.83.

(f) Each litre of consumed O_2 produces different amounts of kcal at different non-protein RQs, because that O_2 is used in oxidizing a specific proportion of carbohydrates and fats at a given non-protein RQ; e.g., at non-protein RQs of 1, 0.83 and 0.71, each litre of consumed O_2 produces 5.047, 4.838 and 4.690 kcal, respectively. Such *calorie equivalents* of O_2 at respective RQs can be used in BMR measurements from respiratory gas exchanges.

(g) RQ normally approaches 1.00 immediately after food intake, because chiefly the absorbed glucose is oxidized at this stage. But 10 hours after a meal or in diabetes or fasting, RQ falls to nearly 0.7 as fat is the principal nutrient then oxidized and lipogenesis from carbohydrates is also reduced.

(h) During heavy exercise, increased tissue metabolism and enhanced pulmonary ventilation increase the CO_2 output; but O_2 consumption does not increase proportionately as the muscles can work with considerable O_2 -debt. So, RQ may far exceed 1.00 during heavy work.

15.3 BASAL METABOLIC RATE

Basal metabolic rate or BMR is the amount of energy produced by a person at complete physical and mental rest, lying comfortably awake in a thermoneutral environment and in the postabsorptive state (i.e., 12-14 hrs after the last meal). BMR includes the energy spent in essential activities like heart beats, respiratory movements, renal functions, muscle tone and transmembrane ion-transport by sodium pump; but it excludes any energy spent in digestion and specific dynamic action.

Indirect calorimetry for BMR

Indirect calorimetry is based on the calorific equivalent of the volume of O_2 consumed by the person at the non-protein RQ, calculated from his respiratory gas exchange and urinary NPN during the given interval.

Benedict-Roth spirometer is ordinarily used for indirect calorimetry. An inverted hanging cylinder, called the spirometer bell, is

counterbalanced over a pulley by a weight carrying a pen which writes on a revolving kymograph drum (Fig. 15.1). The open lower end of the spirometer bell dips into water in the annular space between two other concentric cylinders. The closed space, enclosed by the three cylinders, is filled with O_2 and contains a soda-lime tower with a one-way valve. This space is connected to a mouthpiece through a 3-way valve and two tubes, one of the latter carrying another one-way valve. The mouthpiece is introduced into the patient's mouth and his nostrils are closed by a nose-clip. By working the 3-way valve, the patient is then connected to the spirometer and allowed to respire from and into it, usually for 6 minutes, the one-way valves directing the gas to circulate in a single direction. With the progressive consumption of O_2 and absorption of the expired CO_2 by the soda-lime tower, the bell gradually sinks and the counterweight pen floats up. The volume of O_2 consumed is obtained from

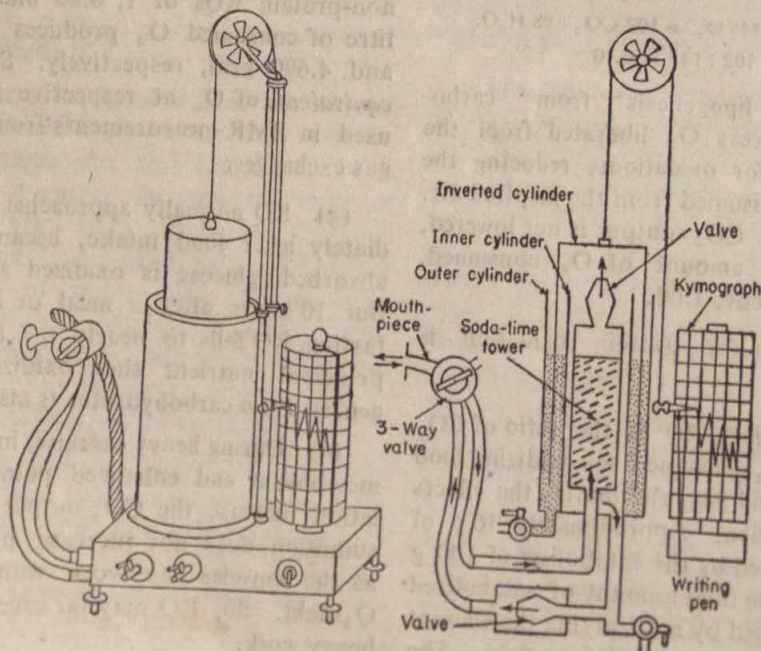


Fig. 15.1. Benedict-Roth spirometer.

the initial and final levels of the writing pen. The non-protein RQ is then assumed to be 0.83 and the BMR is computed by multiplying the volume of consumed O_2 with the calorie equivalent of O_2 at the RQ of 0.83.

Knipping spirometer (Fig. 15.2) obviates the need to assume the non-protein RQ, by directly estimating both the O_2 consumption and the CO_2 output. Here, the soda-lime tower is replaced by a glass container carrying conc. alkali for absorbing the expired CO_2 . The absorbed CO_2 is later re-evolved by adding H_2SO_4 to the alkali, and its volume is estimated from the rise in the spirometer bell level.

Factors influencing BMR

1. *Body surface area and lean body mass* : BMR is directly proportional to the body

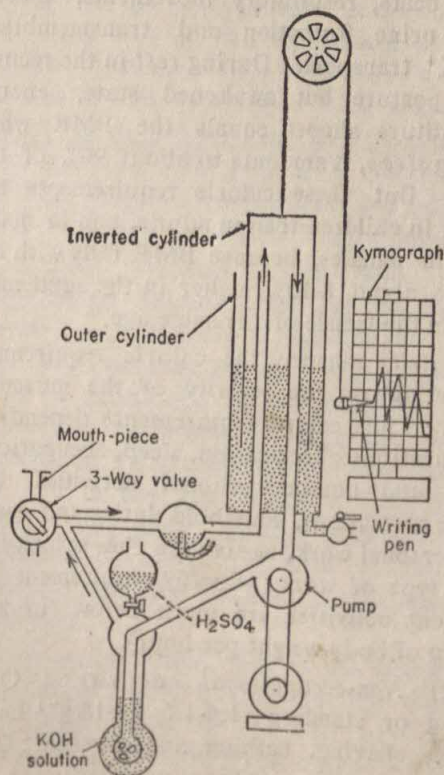


Fig. 15.2. Knipping spirometer.

surface area and is usually expressed in kcal or MJ per sq meter of body surface area per hour ($kcal\ m^{-2}\ hr^{-1}$). Body surface area ($A\ cm^2$) is conveniently calculated in man from height ($H\ cm$), weight ($W\ kg$) and a constant (71.84), using the formula of Dubois and Dubois :

$$A = W^{0.425} \times H^{0.725} \times 71.84$$

Using A computed in cm^2 , the body surface area in m^2 can be easily calculated as $10^{-4}A$. Separate nomograms have been constructed for adults and children on the basis of the above formula, for getting the body surface area directly from height and weight. On the other hand, anthropometric data have been used to formulate simpler and more precise methods for estimating the surface area. For example, the surface area ($A\ cm^2$) may be computed from the height ($H\ cm$) of the body and the circumference ($C\ cm$) at the middle of the thigh : $A = 2HC$.

BMR is closely proportional to the lean body mass. In terms of lean body mass, differences in BMR between man and woman, or fat and lean persons, largely disappear.

2. *Age* : Infants and children have much higher BMR than adults. BMR rises upto $1\frac{1}{2}$ years of age and thenceforth declines rapidly upto adolescence, the decline being steeper in females than in males. After puberty, BMR continues to fall at a much slower rate in both sexes. The decline becomes relatively rapid again in the old age.

3. *Sex* : Expressed in terms of m^2 of body surface area, BMR is higher in males than in females of identical age. Infants of both sexes have almost identical BMR, but girls suffer a steeper decline in BMR with age than boys so that the latter have much higher BMR than the former at adolescence. This difference continues for the rest of the life because the BMR declines with age at the same rate in both sexes. The difference largely

disappears, however, if the BMR is expressed in relation to the lean body mass.

4. *Race and climate* : Tropical races and residents of warmer countries have lower BMR than European races and residents of colder countries, respectively.

5. *Nutritional status* : Starvation reduces BMR by lowering thyroid activity, muscle tone, total cell mass, heart rate and urine secretion.

6. *Hormones* : BMR rises by 20-60% in hyperthyroidism and falls by 20-40% in hypothyroidism. Growth hormone also increases BMR ; its oversecretion raises the BMR in gigantism and acromegaly while its subnormal secretion results in a low BMR in dwarfism. Adrenaline secreted on cold exposure, quickly raises the BMR.

7. *Pregnancy* : BMR rises by about 5% in the first two trimesters and by about 12% in the last trimester of pregnancy. These result from the rise in cell population and in the metabolic rate of both fetal and maternal tissues.

8. *Sympathetico-adrenal status* : Any stimulation of the sympathetico-adrenal system, e.g., in emotions or on cold exposure, increases BMR by enhancing muscle tone, heart rate, respiratory movements and adrenaline secretion.

15.4 SPECIFIC DYNAMIC ACTION

After food intake, heat production rises more than what is expected to be produced by the catabolism of the ingested food. This additional heat output is called the specific dynamic action (SDA) and is about 30% for proteins, 13% for fats, and 5% for carbohydrates. The rise starts within 15 minutes of food intake and may continue up to 6 hours. The heat of SDA can be utilized for body temperature regulation, but not for muscular or cellular work. SDA is diminished during starvation. It is also reduced during growth, pregnancy and convalescence when amino acids

are mainly incorporated in tissue proteins and their catabolism is depressed. SDA of proteins seems to be mainly due to oxidative deamination and urea synthesis, and is substantially reduced by the simultaneous intake of fats with proteins ; carbohydrates also have a similar, though somewhat less, depressing effect on the SDA of proteins. On an ordinary mixed diet, the total calorie requirement should be increased by 10% to provide for SDA.

15.5 CALORIE REQUIREMENTS

Carbohydrates, fats and proteins of food should supply sufficient calories for growth, BMR, tissue maintenance, reproduction, thermoregulation and muscular activity.

Even during complete rest, BMR has to be maintained for ceaseless vital activities like heart beats, respiratory movements, muscle tone, urine formation and transmembrane $\text{Na}^+\text{-K}^+$ transport. During *rest* in the recumbent posture but awakened state, energy expenditure almost equals the BMR while during *sleep*, it amounts to about 90% of the BMR. But these calorie requirements are higher in children than in adults, and in males than in females, because BMR falls with age and is about 8-10% higher in the adult male than in the female of the same age.

Activity enhances the calorie requirement depending on the severity of the muscular work. Thus, calorie requirements depend on the durations of relaxation, sleep, occupational work and non-occupational activities like active recreations, household duties and essential personal work, as also on the severity of each type of work. Energy requirements for different activities are given below (in kcal per kg of body weight per hour).

(a) *Non-occupational activity* : Quiet sitting or standing : $1.4\text{-}1.6 \text{ kcal kg}^{-1} \text{ hr}^{-1}$; eating, shaving, bathing and dressing : 2-4 ; walking : 3-4 ; walking upstairs : 15-16 ; cycling : 7-10 ; running : 8.

(b) *Sedentary occupations*: sewing, kitchen work, clerical work, teaching and laboratory work: 1.5-3.4.

(c) *Moderate activities*: plumbers, transport workers, postmen, furniture painters, shoemakers, peasants, tennis players, cricket players and laundry workers: 3.5-5.8.

(d) *Heavy activities*: stone masons, coal miners, weightlifters, soccer players, swimmers, mountaineers, lumbermen, porters carrying heavy loads, and rickshaw pullers: 6-10.

During *growth*, the calorie requirement is high due to the higher BMR per kg of body weight in infants and children, higher energy expenditure in active movements and play, and the need for additional nutrients for growing tissues.

Aging reduces the calorie need progressively due to a progressive decline in BMR with age and a fall in physical activities. Above 50 years of age, BMR falls on average by 7.5% in each 10 years in both sexes. The total calorie requirement may consequently decrease from 2800 kcal at 35 years of age to 2000 kcal at 75 in the male, and from 2200 kcal at 35 to 1550 kcal at 75 in the female.

During *pregnancy*, the daily calorie requirement increases for the growth and development of maternal reproductive organs and fetal tissues, for the deposition of upto 3 kg of additional fat to be utilized during subsequent lactation, and also for the rise in BMR by 5% in the first two trimesters and by 12% in the last trimester of pregnancy. Thus, the daily calorie requirement is enhanced by about 120 kcal in the first trimester and by 300 kcal in the last two trimesters.

During *lactation*, daily calorie requirement is increased by 550 kcal in the first 6 months of lactation and by 400 kcal in the next 6 months, to provide for an optimal daily milk output of 850 ml.

The total calorie need should be enhanced by 10% to provide for SDA.

15.6 ADULT CONSUMPTION UNIT

In assessing the dietary calorie requirements of families, tribes, socioeconomic groups or an entire population, adult consumption units are used to express the calorie needs of individuals differing in age, sex and occupations. Adult consumption unit (ACU) expresses the daily calorie requirement of a person in terms of the average calorie need of a reference man, viz., a normal sedentary adult man. The calorie requirement of the latter, estimated as 2400 kcal in India, is accepted as 1 ACU. Following ACUs are proposed by ICMR for Indians.

1. Adult man		3. Adolescent	
sedentary	=1.0	12-21 yrs	=1.0
moderate worker	=1.2	4. Children	
heavy worker	=1.6	1-3 yrs	=0.4
2. Adult woman		3-5 yrs	=0.5
sedentary	=0.8	5-7 yrs	=0.6
moderate worker	=0.9	7-9 yrs	=0.7
heavy worker	=1.2	9-12 yrs	=0.8

The daily calorie requirement of a 4-member family is computed in ACU as follows.

Man, heavy worker	=1.6	1 ACU	=2400 kcal
Woman, moderate worker	=0.9	3.8 ACU	=3.8 × 2400
Boy, 11 years	=0.8		=9120 kcal
Girl, 4 years	=0.5		
Total ACU	=3.8	Calorie requirement of the family	=9120 kcal

15.7 PROTEINS IN NUTRITION

Protein quality

The nutritive value of a protein depends on its digestibility and essential amino acid content (page 356). A protein, containing adequate and balanced amounts of all the essential amino acids, has a high nutritive value because its amino acids are promptly

incorporated into proteins and retained in the body. If a protein is deficient in one or more of the essential amino acids, much of its amino acids cannot be readily utilized for protein synthesis and are consequently catabolized. The essential amino acid, most deficient in a protein, is called its *limiting amino acid*, because it limits the extent to which a food protein can contribute to the synthesis of tissue proteins. Milk, egg and flesh proteins are rich in the essential amino acids; but those of cereals, legumes (pulses), nuts and oilseeds are deficient in some essential amino acids. The limiting amino acids of wheat, sorghum, pulses and gelatin are lysine, tryptophan, methionine and tryptophan, respectively. The nutritive value of a protein is expressed in four ways:

(a) *Digestibility coefficient* (DC) is that percentage of food N which is absorbed from the alimentary canal. The fecal N of an animal is estimated after keeping it on a protein-free diet for a couple of days; this gives the metabolic fecal N coming from body proteins. The animal is then fed with a measured amount of the test protein and the fecal N is estimated again.

$$DC = \frac{\text{food N} - (\text{fecal N} - \text{metabolic fecal N})}{\text{food N}} \times 100$$

Legume and seed proteins have lower DCs than milk and egg proteins.

(b) *Protein score* of a protein is the ratio of the amount of the limiting amino acid in it to the required amount of the same amino acid. Practically, it is given by the *chemical score* of the protein, which is the ratio of the amount of its limiting amino acid to the amount of that amino acid in the egg protein.

(c) *Essential amino acid index*: See page 356.

Both the protein score and the essential amino acid index are used to express the nutritional quality of a protein in terms of its essential amino acids. But neither may be a

true measure of the protein nutritional value because some amount of an essential amino acid in a protein may fail to be released by digestion and to be utilized biologically.

(d) *Biological value* (BV) is the percentage of absorbed N retained in the body. The animal is kept on a protein-free diet for a couple of days and its fecal and urinary nitrogens are estimated to get the amounts of metabolic fecal N and endogenous urinary N, respectively. It is then fed with a measured amount of the test protein and the fecal and urinary nitrogens are determined again.

$$BV = \frac{\text{food N} - (\text{fecal N} - \text{metabolic fecal N})}{\text{food N} - (\text{fecal N} - \text{metabolic fecal N})} \times 100$$

A protein of high BV (e.g., ovovitellin of egg yolk and lactalbumin of milk) contains adequate amounts of all the essential amino acids and is so digested and absorbed that its essential amino acids enter the blood at relative rates optimum for their incorporation into peptides. Proteins of egg, milk, fish, meat, wheat, gelatin and maize have BVs of 96, 84, 76, 74, 65, 63 and 60, respectively. Relatively poor BVs of cereals, gelatins, pulses and oilseeds are due to their deficiency in some essential amino acids. A protein of high BV maintains the N balance while a negative N balance results from the intake of a solitary protein of low BV. Cooking or food processing sometimes improves the BV by changing favourably the relative absorption rates of its essential amino acids from the intestine; heat treatment of soyabean improves its BV by enhancing the absorption of methionine from it.

(e) *Net protein utilization* (NPU) is the percentage of food N retained in the body. It depends on the digestibility and the essential amino acid content of the protein; it is given by the product of biological value and digestibility: $NPU = BV \times DC/100$. Milk, egg, wheat, maize and rice proteins have NPUs of

about 90, 87, 49, 36 and 63, respectively, in children. With the mixed protein content of Indian diet, largely of vegetable origin, the average NPU amounts to about 65.

(f) *Protein efficiency ratio* (PER) is the increase in weight of a growing animal per unit weight of protein consumed during the period for which the gain in body weight has been measured. Egg, milk, fish, meat, rice and wheat proteins have PERs of 3.9, 3.5, 3.1, 2.3, 2.2 and 1.5, respectively. Even a protein that can maintain the nitrogen balance, may have a PER of 0 if it does not promote body growth.

Supplementary action of proteins

The BV of poor quality proteins may be substantially enhanced to maintain N balance and growth, if a mixture of several such proteins is eaten simultaneously; because in such cases, the deficiency of an essential amino acid in one of the proteins is compensated by the adequate amount of the same amino acid in another, and vice versa. This purpose is served by including a small amount of an animal protein of high BV with cheaper plant proteins of poor BV. This emphasizes the advisability of eating mixed foods with more than one item of protein, e.g., idli-sambar, sambar-rice, dosa-sambar, khichdi, dahi-vada, alu-matar and dal-roti. For an optimal supplementary action, pulses (legumes) and cereals should supply proteins in the ratio of 1:4 to 1:5 in the Indian diet.

Protein spacers

Dietary carbohydrates and fats may reduce the protein requirement, because (i) carbohydrates and fats spare proteins from catabolism for energy production, (ii) carbohydrate metabolites like pyruvate, α -keto-glutarate and oxaloacetate provide carbon skeletons for the synthesis of non-essential amino acids, and (iii) dietary supply of carbohydrates reduces the necessity for gluconeogenesis from proteins.

Dietary requirements

Protein allowances should be formulated, keeping in view the average NPU of about 65 for a mixed Indian diet. *Normal adults* need dietary proteins to maintain their N balance by replacing the tissue proteins regularly catabolized. Protein requirement is enhanced by an inadequacy of dietary carbohydrates and fats, poor digestibility of food proteins, impaired intestinal absorption or a deficiency of any essential amino acid. *Stress* increases the requirement by exciting the secretion of glucocorticoids which enhance protein catabolism. Protein requirement rises by about 14 g day⁻¹ in the last two trimesters of *pregnancy* to provide for (i) tissue proteins of the fetus, placenta and enlarging maternal organs, and (ii) plasma proteins in the expanding blood volume of the mother; proteins are laid down mainly in the maternal tissues in the first half of pregnancy, but mainly in the fetal tissues in the later half. Protein requirement rises by about 25 g day⁻¹ in the first 6 months of *lactation* to replace about 10 g of proteins secreted in milk every day and also to maintain the enlarged mammary glands. *Infants* and *children* have considerably higher protein requirements per kg of their body weight, compared to adults, because large amounts of proteins are deposited in the growing tissues. In spite of a decline in calorie requirement in *aged people*, their protein requirement continues almost unchanged to provide for the replenishment of decaying tissues.

Following daily protein allowances have been recommended by ICMR :

Adult woman : 45 g	Adult man : 55 g
Pregnant woman : 60 g	School-going
Lactating woman : 70 g	children : 22-52 g

Following amounts of proteins should be normally obtained from food per kg of body weight per day :

Adult woman : 1 g	Infant : 1.8-2.0 g
Adult man : 1 g	Child : 1.2-1.8 g

Dietary requirements of individual essential amino acids per kg of food proteins are given on page 356.

About 30% or more of the protein requirement should preferably be supplied by animal proteins of high BV to ensure an adequate supply of all the essential amino acids. Ordinarily, 20-25% of the total N requirement should be provided by the essential amino acids of food.

15.8 LIPIDS IN NUTRITION

Fat quality

Triglycerides of short-chain, medium-chain and polyunsaturated fatty acids have higher digestibilities than those of long-chain saturated fatty acids; triglycerides of caprylic (C_8) and palmitic (C_{16}) acids, for example, possess digestibilities of about 97% and 73%, respectively. Absorption of unsaturated triglycerides is also far more rapid than that of saturated ones. Human intestine absorbs 96% of mustard oil, but less than 86% of more saturated coconut oil.

Polyunsaturated fatty acids lower blood cholesterol while saturated long-chain fatty acids like palmitic (C_{16}) and stearic (C_{18}) acids raise the serum cholesterol and plasma triacylglycerol levels. *Essential fatty acids* (EFA) must be adequate in amount in the fat to prevent *phrynodema* (pages 421-423). The requirement for EFA is enhanced by a rise in dietary cholesterol and saturated or *cis*-mono-unsaturated fatty acids. The unsaturated vegetable oils are thus preferable to saturated fats like butter, 'ghee', hydrogenated vegetable products or bacon and beef fats. Not more than 10% of dietary calories should come from saturated fatty acids while polyunsaturated fatty acids may supply even 20% or more of the total calories.

Dietary requirements

Dietary fat should supply about 15-20% of the total calorie requirement—a man requiring

2800 kcal day⁻¹ should take about 62 g of fat, equivalent to 560 kcal. About $\frac{2}{3}$ ths or less of this total fat requirement are supplied by invisible fats present in cereals, legumes, soyabeans, nuts, oilseeds, milk, meat, fish, potato and vegetables while the remainder should come from visible sources of fat including vegetable oils, butter and 'ghee'. More than 40% of dietary calories comes from fat in the USA and Britain; certain Indian tribes thrive well even with less than 2% of their dietary calories coming from fat. Consumption of fats supplying more than 30% of the total calories may elevate the blood cholesterol in sedentary and moderately active persons. But fat requirement increases progressively with the rise in total calorie requirement. In *athletes* and *hard labourers*, upto 45% of total calories may come from fat to reduce the bulk of food necessary for the high calorie need, because fat has a much higher calorie value than proteins and carbohydrates; on the other hand, heavy muscular work prevents the rise in serum cholesterol and lipoproteins, otherwise produced by the high-fat diet.

Fat should be reduced in the diets of *sedentary or obese persons* and in patients of hypercholesterolemia and arteriosclerosis. However, low-fat diets may decrease the availability of fat-soluble vitamins which remain with and are absorbed with food lipids.

In *children*, 25% of the total calorie need should be supplied by dietary fats and a ratio of 2 : 3 should be maintained between invisible and visible fats. An infant, deprived of mother's milk, should be provided with 25 g of fat daily, because normally he gets more than half of his calorie need from the milk fat.

The diet of normal adults should contain EFA equivalent to 3% of the total calories. Because fetal tissues like brain and liver carry EFA-rich lipids, 4.5% of the total calories should be supplied by dietary EFA in pregnant women. The EFA requirement rises to 6% of

the total dietary calories in lactating women because about 4 g of EFA are secreted in the daily milk output. In infants and children, 6% of the total calories should come from EFA. Invisible fats of cereals, legumes, etc., may supply EFA equivalent to 4-5% of the dietary calories. Cholesterol should not exceed 300 mg in the daily food to prevent hypercholesterolemia.

15.9 CARBOHYDRATES IN NUTRITION

Daily requirements

60-75% of the total calories should come from carbohydrates. The energy derived from carbohydrates should not preferably exceed 75% of the total calorie requirement and to prevent ketosis, should not fall below 20% of the total calories. However, poorer people of Southeast Asia, Bangladesh and India often derive more than $\frac{4}{5}$ ths of the dietary calories from carbohydrates.

About 55-65% of the total calories should normally come from easily digestible macromolecular polysaccharides like starches, and not more than 10-15% from mono- and disaccharides like sucrose, lactose and fructose. ICMR recommendations restrict the intake of refined carbohydrates like sugar and jaggery to 5% of the total calories.

Relation with activity

Athletes and heavy workers should take high-carbohydrate high-fat diets to provide large amounts of calories and to prevent depletion of muscle glycogen. A high-carbohydrate diet may enhance the work capacity of muscles and delay fatigue during sustained heavy exercise. Carbohydrates should supply a higher percentage of dietary calories to mountaineers at high altitudes than at the sea-level, because the oxidation of carbohydrates produces more calories per litre of O_2 consumed, compared to non-carbohydrates.

15.10 PROTEIN-ENERGY MALNUTRITION

Protein-energy malnutritions (PEM) occur in India, Bangladesh, Burma and Southeast Asian, West African and Arab countries.

Marasmus

Infants below one year may suffer from marasmus due to a simultaneous deficiency of proteins and food calories. It is common in infants of poor parents when breast feeding is prematurely replaced by less nutritive foods, low in proteins and calories. Diarrhoea and gastroenteritis due to food-borne infections may aggravate the deficiency by reducing digestion and absorption.

The infant gets extremely emaciated. Atrophy and severe wasting of muscles cause thinning of limbs and abdominal muscles, and make the ribs prominent. Hepatic cirrhosis may occur without fatty infiltration. Atrophy of intestinal mucosa and pancreatic acini causes a lack of pancreatic and intestinal enzymes like trypsin, sucrase and lactase; severe impairment of digestion leads to diarrhoea and flatulence. Growth rate, brain weight, BMR and body temperature are lowered. Skin becomes thin, dry, wrinkled and almost devoid of subcutaneous fat; but unlike kwashiorkor, there is no edema or skin lesion like pigmentation, ulcer and desquamation. Liver lipid content and hypoalbuminemia are much less in marasmus than in kwashiorkor. Anemia and hypoglycemia may occur.

Kwashiorkor

Older children may suffer from kwashiorkor due to protein deficiency unaccompanied by calorie deficiency. It is often precipitated by a replacement of breast feeding with low-protein high-carbohydrate foods.

Unlike marasmus, skin lesions and extensive edema are prominent symptoms in kwashiorkor. Patches of hyperpigmentation, exfoliation, desquamation and ulceration are

seen in the skin of particularly buttocks, perineum and legs; but unlike marasmus, some subcutaneous fat persists. Loss of proteins causes atrophy and thinning of muscles, growth failure and weight loss. But the body looks less emaciated than in marasmus, due to extensive edema caused partly by a fall in serum albumin. Like marasmus, atrophy of pancreatic acini and intestinal mucosa impairs digestion and absorption, leading to diarrhoea. Hypoglycemia, anemia, anorexia, depigmentation and loss of hair, low BMR and fall in body temperature are observed. Unlike marasmus, fatty liver and fall in plasma levels of cholesterol, triglycerides and β -lipoproteins are seen in kwashiorkor. Brain development and mental faculties such as initiative, IQ and capacities in visuokinesthetic and audiovisual integration tests, verbal tests and performance tests are depressed.

15.11 STARVATION

1. *Changes in carbohydrate metabolism:*

A couple of days of fasting rapidly uses up tissue glycogens for maintaining the blood sugar and energy production. Consequently, the total carbohydrate reserve may decline by 40% and the blood sugar may reach 40 mg dl⁻¹. Cardiac muscles may, however, continue to have normal glycogen content. Hypoglycemia depresses insulin secretion, but enhances secretions of adrenaline and glucocorticoids; there is a consequent increase in the activities of the key gluconeogenic enzymes like pyruvate carboxylase, FDPase, PEP carboxykinase and glucose-6-phosphatase, and of glycogen phosphorylase, the rate-limiting enzyme for glycogenolysis. These enhance gluconeogenesis and glycogenolysis and maintain the liver and muscle glycogens and the blood sugar, though at a subnormal level. Gluconeogenesis from proteins is considerable in the liver and kidneys in the first few weeks, but continues later at a reduced rate mainly in kidneys.

Hepatic glycogenesis is reduced due to a fall in glucokinase activity in consequence of hypoglycemia. Liponeogenesis from carbohydrates is also reduced owing to the fall in the induction of citrate cleavage enzyme, consequent upon the decline in insulin secretion. These factors save some blood sugar for supply to the tissues.

2. *Changes in fat metabolism:* Carbohydrate reserves are insufficient to meet the calorie needs beyond a couple of days. During starvation, enhanced secretions of glucocorticoids and adrenaline increase the activity of the hormone-sensitive lipase of the adipose tissue and consequently mobilize large amounts of adipose tissue fat to the liver for oxidation. This results in a low RQ (≈ 0.73), a rise in the plasma free fatty acids, ketosis and on prolonged fasting, fatty liver. Fatty acid synthesis is reduced, because the elevated plasma levels of long-chain fatty acids inhibit acetyl-CoA carboxylase and reduce the outflow of citrate from mitochondria for cytoplasmic lipogenesis while the decline in insulin secretion prevents the induction of NADPH-generating malic enzyme and glucose-6 phosphate dehydrogenase.

Metabolic changes, outlined above, reduce the body fat by 15% in several weeks, causing severe loss of adipose tissues, consequent displacement of visceral organs, and loose skin folds due to the loss of subcutaneous fat. Unlike the stored fat, complex structural lipids are catabolized and lost only towards the terminal stage of prolonged starvation.

Lipoprotein lipase activity declines in the adipose tissue, but rises in cardiac and skeletal muscles. This restricts fat uptake by the adipose tissue and increases the availability of fatty acids to the muscles.

Starvation ketosis is more pronounced in females than in males, and in children than in adults. Ketosis and ketonuria produce acidosis and enhance urinary ammonia, pulmonary ventilation and CO₂ elimination.

3. *Changes in protein metabolism*: Negative N-balance results from increased protein catabolism for gluconeogenesis and energy production. This is more pronounced after the loss of depot fat. Breakdown of tissue proteins lowers the body protein by more than 25% and the resulting decrease in the cell mass reduces the intracellular fluid volume considerably. Loss of proteins, fats and intracellular water causes severe emaciation and weight loss. Intestine, liver, spleen, heart and muscles atrophy considerably due to protein catabolism. The weight loss of muscles, heart, spleen and liver may even reach 30, 5, 70 and 60 per cent, respectively. Intestinal atrophy severely restricts digestion and absorption. Skin atrophies to get thin, wrinkled, inelastic and shrunken. Striated muscle fibres are reduced in number, mass and tone; their degeneration increases urinary creatine. Hepatic cells suffer extensive breakdown of organelles. Atrophic changes of the heart lower the cardiac output to produce hypotension and circulatory failure. But brain does not show significant atrophy and intellect is not impaired although prolonged starvation may affect personality. Increased protein catabolism lowers the secretions of insulin, thyroxine and gonadotropins, and causes failures of reproduction and lactation.

Urinary NPN and urea are reduced while neutral sulfur and uric acid are increased in urine. Anemia, poor body immunity, low serum albumin and extensive edema result from protein loss.

4. *Changes in water and mineral metabolisms*: On prolonged starvation, cellular disintegration lowers the intracellular K^+ , total body K^+ , and the intracellular fluid volume. Extracellular fluid (ECF) is reduced in the first few days due to increased water loss, but a subsequent decline in the cell mass expands the extracellular compartment where fluid accumulates, producing edema and restoring the ECF volume. Low serum

albumin contributes to the production of edema. Glomerular filtration is reduced and metabolic water is elevated, lowering the need for drinking water. Na^+ is conserved by reducing its urinary elimination, $Na^+ : K^+$ ratio rises in the body, and Na^+ level may be maintained in the ECF.

5. *Basal metabolism*: Declines in muscle tone, thyroid activity, total cell population, heart rate and urine secretion—all lower the BMR and body temperature.

15.12 COMMON INDIAN FOODS

I. Protein foods:

Milk and milk products: Milk supplies the infant with adequate amounts of proteins, fats, lactose, calcium, phosphorus, vitamin A and some B vitamins, but needs supplementation with other foods as the child grows up.

Of the milk proteins, lactalbumin and lactoglobulin have very high biological values. The phosphoprotein casein is higher in amount in the milks of cow (2.8%), buffalo (3.6%) and goat (2.7%) than in human milk (0.5%). Lactalbumin, lactoglobulin and casein occur in human and cow's milks in average proportions of 3 : 5 : 4 and 4 : 1 : 7 respectively. Lactalbumin has the highest digestibility and casein the lowest.

Lactose, the milk sugar, is remarkably high in mare's milk (6%) and human milk (7%). Milk fats occur in fine emulsions, easily digestible even without the aid of bile salts. Unsaturated fatty acids form about 30% of the fatty acids.

Cow's milk contains about 120 mg calcium and 100 mg phosphorus per decilitre (100 ml); the amounts are higher in buffalo's milk, but only about 30-40 mg dl^{-1} in human milk. Calcium occurs as free Ca^{2+} , casein-bound Ca and inorganic salts; phosphorus occurs in casein and as inorganic and organic phosphates. The Ca : P ratio of milk and the lactic acid produced by lactose fermenta-

tion help in Ca^{++} absorption. Milk is poor in iron.

Milk is rich in riboflavin, carotenes and vitamin A. About 30 and 180 μg of riboflavin are present in 100 ml of human and cow's milks, respectively. These milks contain about 50 and 40 μg of vitamin A, respectively. But milk is poor in vitamins C, D and K; cow's milk is often 'fortified' with vitamin D. Thiamin, riboflavin and nicotinamide amount to about 0.05, 0.01 and 0.04 mg dl^{-1} in cow's milk; these are respectively 2, 2.5 and 2 times their amounts in human milk.

Colostrum is the thermocoagulable, viscous, yellow liquid secreted by mammary glands in the first 4-5 days after parturition. It is richer than milk in proteins, vitamins A, D and E, calcium and phosphorus, but poorer in casein, fat and lactose. Of the proteins, thermocoagulable globulins including immunoglobulins are highest in amount, followed by lactalbumin. IgA immunoglobulins come from the maternal blood and are absorbed by the newborn without change, conferring some humoral immunity.

Eggs: Duck's and hen's eggs supply good quality proteins, easily digestible lipids and vitamins. Egg-yolk contains a thermocoagulable phosphoprotein *ovovitellin* of very high biological value. Egg-white proteins include *ovo-albumin* and *ovoglobulin* of good biological values and a thermolabile basic glycoprotein *avidin* which prevents biotin absorption. Egg is the richest source of dietary cholesterol (160 $\text{mg}/100\text{g}$)—cholesterol occurs mostly in the yolk. The yolk is also rich in vitamin A, Ca, P, thiamin, riboflavin, biotin, pantothenate and Fe. Cooking inactivates avidin, delays cholesterol absorption and prevents egg-borne infections like *Salmonella*.

Flesh foods: Fish flesh contains about 14-25% proteins of high biological value. Lean fishes like cod, haddock, 'singhi' (*Hetero-*

pneustes fossilis) and 'magur' (*Clarias batrachus*) contain less than 1% fat and are easily digestible. Oily fishes like hilsa, dhain (*Silonia silondia*), sarputi (*Barbus sarana*) and 'koi' (*Anabas testudineus*) contain 8-20% fat, rich in unsaturated fatty acids. Viscera, liver and oily flesh of fishes are rich in vitamins A and D while sun-dried fish is a good source of vitamin K. Prawn and crab flesh contains lipids and cholesterol. Fish flesh is also rich in phosphorus and B vitamins. Fish bones supply much Ca and P, when chewed to a powder and swallowed.

Meat and liver contain B_1 , B_2 , B_6 , B_{12} , nicotinamide, S, Fe, P and about 18-25% protein of high biological value, but cannot supply carbohydrates because of a rapid glycolysis of all glycogen after slaughter. Lean meat and bacon carry 0.7% and even 40% fat, respectively.

Pulses or dry legumes: These contain 20-25% protein, rich in lysine but deficient in tryptophan and methionine; carbohydrates, mainly starch, vary from 58 to 60%. Bengal gram, unlike others, contains as much as 5.6% fat. Sufficient amounts of Ca, P, Fe, K, thiamin, biotin, nicotinamide and carotenes occur in pulses. Germination enhances carotenes, riboflavin and vitamin C. 'Khesari' (*Lathyrus sativa*) contains β -N-oxalylamino-l-amine which produces pyramidal tract lesions in lower spinal segments, leading to spastic paralysis of legs and paraplegia in flexion (*lathyrism*).

Soyabean: It contains more than 40% protein, rich in lysine but somewhat deficient in methionine. It is also rich in B_1 , B_2 , nicotinamide, carotenes, Ca, P and Fe. 85% of its fatty acids consists of unsaturated acids, linolenic acid forming 7% of the fatty acids.

II. Protective vegetables and fruits:

Their minerals and vitamins prevent nutritional deficiencies.

Green leafy vegetables: These are rich sources of Ca, Fe, carotenes, folate, riboflavin and vitamins K and C. Dark green leaves are generally richer in minerals and vitamins than pale green leaves.

Orange-yellow vegetables: Beet, carrot, etc., are rich sources of carotenes.

Sour fruits: Citrous fruits like lemon, sweet lime (musambi), orange and tomato are good sources of vitamin C. Banana contains about 20-25% carbohydrate and some B and C vitamins.

III. Cereals, roots and tubers :

These supply mainly carbohydrates, and also some proteins, B vitamins, Ca and P.

Rice: Rice contains about 78-79% starch and about 6.5-7% protein, poor in lysine. Rice protein has a higher BV and PER than wheat proteins. Superficial layers and the germ of rice grain are rich in minerals and vitamins, but are largely removed as rice polishings during milling. Some B vitamins and minerals are also lost during washing and cooking. Parboiled rice is richer than raw rice in Ca, P, B₁, B₆ and nicotinamide because parboiling transfers minerals and vitamins to deeper layers of the grain and prevents their milling loss.

Wheat: Wheat contains more proteins than rice, but lacks lysine in its protein. Starch and cellulose form respectively 97% and 3% of the carbohydrates. Superficial layers and the germ are rich in Ca, P, Fe, nicotinamide, B₁, B₂, B₆ and tocopherols. 93-95% of these nutrients occurs in high-extraction flours (atta) and far less in low-extraction flours (maida), because the superficial layers are progressively removed as bran during milling and extraction. But high-extraction flours are richer than low-extraction flours in phytate which interferes with Ca²⁺, PO₄³⁻ and Fe²⁺ absorptions. Flours are often 'fortified' with CaCO₃, ferric ammonium citrate and thiamin.

Potato: It contains 20-22% starch and significant amounts of K⁺ and vitamin C, but is much poorer than cereals in proteins, Ca, Fe, B₁, B₆ and nicotinamide.

IV. Concentrated foods :

These are high-calorie foods, rich in sugars or fats but with little or no water.

Butter and ghee: These contain about 80-82% and 90-99% fat, respectively, and good amounts of carotenes and vitamin A. They carry mostly saturated fats and are deficient in minerals and other vitamins.

Vegetable oils: Oils of groundnut, coconut and oilseeds and hydrogenated vegetable fats (vanaspati) are almost 100% fat. Triglycerides of vegetable oils, except coconut oil, are rich in unsaturated fatty acids including linoleic acid, while hydrogenated products are rich in saturated fatty acids. Oils contain tocopherols, but are deficient in minerals and other vitamins. Hydrogenated vegetable fats are 'fortified' with 50 IU of vitamin D and 700 IU of vitamin A per ounce. Excessive intake of rapeseed oil may lead to myocardial damage due to the accumulation of a fatty acid of that oil in myocardium.

Molasses, sugar and honey: Sugar-cane molasses contain about 60% sucrose and some Ca, Fe, biotin and pantothenate. Cane sugar contains about 99% sucrose. Honey has about 80% carbohydrate, consisting of dextrins, glucose and fructose.

15.13 DIET FOR NORMAL ADULT

Calorie requirement: The daily allowance for calories is calculated on the basis of the BMR of the person and the intensity as well as the duration of his various activities.

(a) Energy expenditure amounts to about 90% of the BMR during sleep, and practically equals the BMR during bed rest in recumbent but awake condition. Because the BMR is 8-10% higher in the adult male than in the

TABLE 15.1. Average values of energy and some nutrients per 100 g of different foods.

Foodstuff	Energy (kcal)	Carbohydrate (g)	Fat (g)	Protein (g)	Calcium (mg)	Iron (mg)
Rice						
parboiled	345	79.2	0.4	6.2	9	3.8
raw	345	78.4	0.5	6.7	10	3.0
flakes	345	77.6	1.0	6.4	20	18.0
puffed	325	73.4	0.2	7.4	22	6.4
Wheat	345	72.0	1.3	11.4	40	5.0
Wheat flour						
high-extraction	340	69.4	1.6	12.0	47	10.5
Low-extraction	350	74.7	0.8	11.0	20	2.0
Semolina (suji)	350	75.4	0.8	10.3	15	1.7
Barley	335	70.0	1.0	11.5	25	2.8
Pulses						
Bengal gram	370	59.4	5.6	20.6	56	9.0
black gram	348	60.0	1.3	24.1	150	9.0
green gram	350	60.3	1.2	24.4	73	8.0
lentil	340	58.8	0.5	25.0	70	4.6
red gram	336	57.8	1.8	22.2	70	5.5
Soyabean	430	20.0	19.8	43.0	235	11.0
Milk						
human	66	7.2	3.5	1.5	35	—
cow	68	4.5	4.0	3.5	120	0.1
buffalo	115	5.0	8.6	4.4	205	0.1
goat	73	4.5	4.4	3.6	165	—
Curd (dahi)	60	3.0	4.0	3.0	148	0.1
Casein (chhana)	264	2.0	19.1	21.0	185	—
Cheese, cheddar	393	2.0	32.0	24.3	725	1.5
Butter	725	0.3	80.2	0.5	20	—
clarified (ghee)	885	—	98.3	—	—	—
'Vanaspati'	900	—	100.0	—	—	—
Vegetable oils	900	—	100.0	—	—	—
Sugar	398	99.4	—	0.1	10	—
Molasses	240	59.6	—	0.4	155	8.5
Honey	320	79.6	—	0.4	5	0.8
Egg, hen's	170	0.5	13.1	13.5	60	2.0
Egg yolk	555	0.5	31.9	16.4	150	7.0
Liver	150	1.4	7.4	19.4	10	6.0
Mutton	192	0.2	13.0	18.6	15	2.5
Fowl	109	0.2	0.5	26.0	24	—
Rohu	95	4.3	1.4	16.4	650	0.8
Hilsa	275	3.0	19.6	21.6	180	2.0
Bherki	80	3.0	0.9	15.0	475	3.0
Koi	160	4.8	9.0	15.0	400	1.2
Puti	105	3.0	2.2	18.2	112	1.0
Sardine	100	—	1.8	21.0	90	2.4
Pomfret	90	2.2	1.4	17.2	200	0.7
Prawn	88	2.5	0.7	20.0	320	5.0
Potato	94	21.5	0.1	1.8	10	0.6
Sweet potato	120	28.4	0.2	1.2	40	0.8
Tapioca	156	38.5	0.1	0.3	48	0.8

TABLE 15.1. *Continued.*

Foodstuff	Energy (kcal)	Carbohydrate (g)	Fat (g)	Protein (g)	Calcium (mg)	Iron (mg)
Beet root	45	9.5	0.1	1.5	18	0.9
Colocasia	94	21.0	0.1	2.3	38	1.5
Carrot	50	11.0	0.2	1.0	80	2.0
Onion	50	11.2	0.1	1.1	50	0.8
Brinjal	24	3.9	0.4	1.3	17	0.8
Cauliflower	30	4.3	0.3	2.5	35	1.4
Cabbage	26	4.8	0.1	1.5	40	0.7
Spinach	25	3.0	0.5	2.1	72	11.0
Ipomoea leaves	27	3.4	0.3	2.5	110	4.0
Amaranth leaves	45	6.0	0.6	4.0	400	25.4
Coriander leaves	44	6.0	0.7	3.4	185	19.0
Tomato, ripe	20	3.8	0.2	0.8	47	0.3
Banana, ripe	110	26.1	0.2	1.0	15	0.6
Orange	50	11.3	0.2	0.8	25	0.2
Lemon	55	11.0	0.8	0.9	70	2.0
Mango, ripe	70	15.8	0.5	0.5	12	0.8

female of the same age, the energy cost for identical periods of sleep or bed rest is higher in a man than in a woman.

(b) Non-occupational activities include essential personal work like shaving, dressing, bathing and eating, household chores, climbing stairs, recreations ranging from card playing to active athletics, reading, talking and journey between the residence and the office. Energy expenditure in such non-occupational activities depends upon the severity of muscular work and the duration of each activity.

(c) The severity and duration of occupational or professional work must also be considered in computing calorie requirements. Sedentary or light occupations like those of typists, clerks, tailors, teachers, housewives, waiters and electricians require light activities with energy expenditure varying from 1.5 to 3.4 kcal per kg of body weight and per hour of activity. Professions like those of peasants, postmen, transport workers, furniture painters, washermen and cricket players involve moderate degrees of muscular work to require 3.5-5.8 kcal kg⁻¹ hr⁻¹. Stone masons, lumbermen, coal miners, rickshaw pullers,

weightlifters, soccer players and mountaineers perform heavy muscular work spending 6-10 kcal kg⁻¹ hr⁻¹.

(d) The total calorie need should be enhanced by 10% to provide for the specific dynamic action.

Table 15.2 gives the computation of calorie and nutrient requirements of a typical normal adult man.

Protein requirement: Adequate amounts of proteins should be provided to maintain the N-balance by replacing the catabolized tissue proteins. In view of a significantly greater loss of endogenous N in sweat in the tropical climate, the obligatory loss of endogenous N has been put at 2.5 mg per basal calorie per day for Indians by the ICMR. Besides, the NPU for ordinary mixed Indian diets, largely of vegetable origin, has been estimated as 65. Considering these facts, ICMR has recommended a dietary intake of 1 g of protein per kg of body weight per day for adult Indians. Preferably, 1/3rd of dietary proteins should be supplied by milk, egg, flesh and liver proteins having high NPU and biological values and containing rich amounts

of B vitamins. The requirement of costly animal proteins may be reduced by a judicious intake of cereal-pulses, cereal-soyabean or cereal-potato-pea mixtures, utilizing their supplementary actions in enhancing their biological values. Pulses are good sources of not only proteins but also carotenes and vitamins. For optimal supplementary actions, pulses and cereals should supply proteins in the ratio of 1 : 4 to 1 : 5.

Fat requirement : About 20% of the total calories should be supplied by fat ; the ratio of fats to non-fats should rise with the rise in calorie requirements and muscular work. However, visible fats and oils must not exceed 15% of the total calories ; the remainder should be supplied as invisible fats in cereals,

pulses, milk, flesh foods and vegetables. Excess of saturated fats should be avoided as they may raise the serum cholesterol and triacylglycerol levels. Not more than 10% of the total calories should come from saturated fatty acids. 20% or more of the dietary calories may be supplied by polyunsaturated fatty acids ; essential fatty acids should supply at least 3% of the total calories. Consumption of vegetable oils of low melting points may supply adequate amounts of unsaturated fatty acids and some vitamin E. Some saturated fats like butter should be included to supply fat-soluble vitamins.

Carbohydrate requirement : 60-75% of the total calories should be supplied by carbohydrates, of which 80-85% should consist of

TABLE 15.2. *Computation of calorie and nutrient requirements.*

Subject : Adult male (25 yrs). Weight : 55 kg. BMR : 60 kcal hr⁻¹.

I. *Calorie requirement*

Activities		kcal
1. Sleep for 8 hrs @ 90% of BMR	$= 60 \times 0.9 \times 8$	= 432
2. Non-occupational activities :		
(a) Eating, shaving, bathing and dressing @ 2 kcal/kg/hr for 1.5 hrs	$= 2 \times 55 \times 1.5$	= 165
(b) Quiet sitting or standing @ 1.4 kcal/kg/hr for 1 hr	$= 1.4 \times 55 \times 1$	= 77
(c) Writing, card-playing, gossiping and playing musical instrument @ 1.5 kcal/kg/hr for 6 hrs	$= 1.5 \times 55 \times 6$	= 495
(d) Walking @ 3 kcal/kg/hr for 1 hr	$= 3 \times 55 \times 1$	= 165
3. Occupational work @ 3.5 kcal/kg/hr for 6.5 hrs	$= 3.5 \times 55 \times 6.5$	= 1251
		2585
10% allowance for SDA		259
		2844
Total daily calorie requirement		≈ 2840

II. *Nutrient requirements*

Nutrients

	Amounts	kcal
1. Proteins @ 1 g/kg (1 g protein ≡ 4 kcal)	$55 \times 1 = 55 \text{ g}$	220
2. Fats @ 20% of total calories (1 g fat ≡ 9 kcal)	$\frac{2840 \times 0.20}{9} = 63 \text{ g}$	567
3. Carbohydrates for the balance of calories (1 g carbohydrate ≡ 4 kcal)	$\frac{2840 - (220 + 567)}{4} = 514 \text{ g}$	2056
	Total	2843

TABLE 15.3. *Average daily dietary requirements of energy, proteins, fats and minerals (largely on 1981 recommendations of ICMR).*

<i>Individuals</i>	<i>Energy (kcal)</i>	<i>Protein (g)</i>	<i>Fat (g)</i>	<i>Ca ()</i>	<i>Fe (mg)</i>	<i>I (µg)</i>
Man, 25 yrs, 55 kg						
sedentary	2400	55	53	0.5	25	130
moderate activity	2800	55	62	0.5	25	140
heavy activity	3900	55	150	0.5	25	140
Man, 60 yrs, 55 kg	2250	55	38	0.5	25	110
Woman, 25 yrs, 45 kg						
sedentary	1500	45	42	0.5	30	100
moderate activity	2200	45	49	0.5	30	100
pregnant*	2500	60	55	1.0	40	125
lactating†	2750	70	60	1.0	30	150
Infant, 6-12 mths	760	12	42	0.5-0.6	7	40
Child, 1-3 yrs	1200	22	40	0.4-0.5	20-25	60
4-6 yrs	1700	30	47			80
7-9 yrs	2050	35	57			100

* 2nd-3rd trimester. † First 6 months.

digestible macromolecular polysaccharides like starches. ICMR restricts the allowance of refined disaccharides like sugar and jaggery to 5% of the total calories. A high proportion of dietary starches should come from cereals which simultaneously supply B vitamins and calcium. A high-carbohydrate high-fat diet is preferred for athletes and heavy workers with high calorie needs; a high-carbohydrate diet prevents depletion of muscle glycogen, enhances work capacity and delays fatigue during sustained heavy work. Mountaineers at high altitudes should also take high-carbohydrate diets, because carbohydrate oxidation produces more calories per litre of O₂ consumed, compared to non-carbohydrates.

Calcium, phosphorus and vitamin D requirements: About 0.5 g of calcium and a similar amount of phosphorus should be supplied in the daily diet of adults to replace their loss in urine, bile and sweat. Cereals (particularly 'ragi'), milk, carrot, bony fish and leafy vegetables are sources of calcium. Adequate intake of proteins fulfils the phosphorus requirement also. About 100 IU of vitamin D should be required daily by adult

men and women. Fish viscera and oily flesh of fish may be occasionally taken for vitamin D; but regular dietary supply of the latter is not essential in normal adults of tropical countries as sufficient amounts may be synthesized in the skin in presence of sunlight. However, regular supplementation with 100 IU of vitamin D is advisable for women residing in dark tenement houses or observing 'purdah', and for men working regularly in nightshift industries, subterranean mines, newspaper press and submarines.

Iron requirement: The daily basal loss of iron through gastrointestinal tract, urine and sweat averages about 0.7 mg in adults. Menstrual loss in women causes an additional average daily loss of about 0.6-1 mg. Allowing for an intestinal absorption of 3% and 5% of food iron in normal man and woman respectively, the daily dietary iron requirements may be fixed at about 25 mg for adult man and about 30 mg for adult woman. Liver, lentil and lettuce are good sources of iron.

Requirements for vitamin A and other vitamins: ICMR has recommended a daily

intake of 750 μg of vitamin A to prevent any impairment of scotopic vision and the abnormalities of electroretinogram. This may be supplied by providing adequate amounts of milk, dark-green leaves and yellow-red vegetables in the daily dietary. Leafy vegetables are also good sources of folate, riboflavin and vitamin K. Inclusion of sour fruits ensures the supply of the daily requirement of about 40 mg of vitamin C.

15.14 DIET FOR PREGNANT WOMAN

Calorie requirement: Requirement for dietary calories rises during pregnancy, particularly in its later half, because of (a) the rise in BMR by about 5% in the first two trimesters and by 12% in the last trimester, (b) increased requirements for the growth and development of fetus, placenta and maternal reproductive organs like uterus and mammary glands, (c) the need to provide for increased hemopoiesis in the mother, (d) increased muscular work in carrying the rising weight of the fetus during each movement, and (e) the need to provide for the deposition of 2-3 kg of fat in the maternal tissues for utilization in the subsequent lactation. In view of these

enhanced needs, ICMR has recommended an additional daily allowance of 120 kcal in the first trimester and 300 kcal in the last two trimesters of pregnancy. However, restriction of physical activity in the later half of pregnancy may partly reduce this additional need.

Protein requirement: This rises by about 15 g per day in the last two trimesters to provide mainly for (a) the formation of proteins in growing fetal tissues and maternal reproductive organs, and (b) synthesis of plasma proteins for the enhanced blood volume of the mother. Of the total daily requirement of 60 g proteins, about $\frac{1}{4}$ th should be supplied by animal proteins of high biological values like milk, egg and flesh proteins.

Fat requirement: To supply essential fatty acids for the tissue lipids of fetal brain and liver, the food should include essential fatty acids providing 4.5% the total calorie need.

Calcium, phosphorus and vitamin D requirements: About 30 g of calcium are deposited in the fetal tissues during the entire period of pregnancy; this is mostly supplied in the third trimester from the maternal blood

TABLE 15.4. Some balanced diets. (Amounts in g day⁻¹.)

Food items	Moderately active man	Moderately active woman			Child (4-6 yrs)
		Normal	Pregnant	Lactating	
Rice	500	400	475	500	250
Lentil	50	40	40	45	30
Milk	200	200	250	250	250
Fish	30	30	30	50	30
Egg	50	50	50	50	30
Sugar	35	25	30	30	30
Potato	60	40	50	50	25
Butter	15	10	10	15	15
Vegetable oil	30	20	20	25	18
Leafy vegetables	50	80	80	100	40
Other vegetables	60	40	40	40	25
Citrous fruit	20	20	20	20	10
kcal	2800	2200	2500	2750	1700

at the average rate of about 300 mg day⁻¹. So, the daily need for calcium is enhanced by 300 mg in the last trimester. A total daily allowance of about 1 g each of Ca and P and about 200 IU of vitamin D seems to be a safe recommendation for the later half of pregnancy; otherwise, the maternal bones may get decalcified to supply the minerals to the fetus, resulting in osteomalacia.

Iron requirement: During the entire period of pregnancy, about 200-350 mg of iron are deposited in fetal tissues and utilized in forming fetal blood, and about 400-600 mg of iron are needed for forming additional amounts of maternal hemoglobin. Besides, about 90 mg of iron will be lost during parturition. These put the iron requirement at about 3.5 mg day⁻¹ during pregnancy; as about 8% of the food iron is absorbed during pregnancy, the iron requirement should be met by a daily allowance of 40 mg of iron throughout pregnancy. Iron-rich foods or supplementation with ferrous sulfate are recommended; otherwise, iron deficiency may lead to microcytic hypochromic anemia of the mother, low fetal stores of iron and poor fetal growth.

Folate and cobalamin requirements: The daily allowances for folic acid and vitamin B₁₂ should be 0.3 mg and 1.5 µg respectively, to help in the nucleic acid synthesis in proliferating tissues and developing RBC in both mother and fetus. A deficiency of either of these vitamins may produce megaloblastic or macrocytic anemia in the mother.

Requirements for vitamins K, C, A and B complex: In the last trimester of pregnancy, an additional daily amount of 3 IU of vitamin K should be provided as a protection against excessive bleeding during parturition and also to build up fetal stores of vitamin K and to synthesize Ca²⁺-binding proteins in the fetal bones. Daily allowances for thiamin, riboflavin and nicotinamide amount to 1.3, 1.5 and 17 mg, respectively, because their require-

ments rise with the amount of dietary calories. Due to the enhanced allowance for proteins, daily requirement for pyridoxine is also increased to 2.5 mg. Although no additional allowance for vitamins A and C has been recommended by the ICMR, the Food and Nutrition Board of the National Research Council (USA) has recommended daily additional intakes of respectively 1000 IU and 15 mg of those two vitamins, because vitamin A is involved in mucopolysaccharide synthesis and vitamin C in collagen synthesis in the growing fetal tissues.

The daily diet of the pregnant woman should include adequate amounts of milk for supplying calcium, carotenes, B vitamins, proteins and fluids, oily fishes and liver oils for vitamins A and D, dark green leaves for carotenes, vitamin K, B vitamins and iron, and a sour fruit for supplying vitamin C. A prescribed diet for a pregnant woman is given in Table 15.4.

15.15 DIET FOR LACTATING WOMAN

Calorie requirement: Daily allowance for total food calories should be enhanced by 550 kcal in the first 6 months of lactation and by 400 kcal in the next 6 months, to provide the raw materials for milk ingredients and the energy for lactation; this is based on the estimated 80% efficiency of conversion of dietary calories and the optimal daily milk output of 850 ml. A part of the additional calorie need is also supplied by the fat deposited during pregnancy.

Protein requirement: Daily protein allowance should be enhanced by 25 g in the first 6 months for maintaining the secreting mammary tissue and for replacing the daily loss of about 10-12 g of proteins in milk. About 1/4th of total proteins should preferably be supplied in the form of animal proteins of high BV.

Fat requirement: To replace about 4 g of essential fatty acids secreted in the milk every

day, the food should contain adequate amounts of EFA to give about 6% of the total calorie need in the first 6 months and about 4.5% thereafter. This is easily provided by a corresponding enhancement of vegetable oils in the diet.

Calcium, phosphorus and vitamin D requirements: About 250-300 mg of calcium and corresponding amounts of phosphorus are secreted in the daily milk output. To replace them, 1 g each of calcium and phosphorus should be provided in the daily diet. 200 IU of vitamin D should also be available in the daily diet to serve in the intestinal absorption and retention of those minerals. Deficiency of these nutrients may cause the drainage of calcium and phosphorus from maternal bones for secretion in the milk and may consequently produce osteomalacia.

Iron requirement: About 1 mg of iron is secreted in the daily milk output. But this additional loss of iron is more than compensated by the absence of menstrual loss of iron due to lactational amenorrhoea. Thus, iron requirement is not enhanced above the non-pregnant level.

Requirements for vitamins A, C and B complex: To compensate for the daily loss of vitamins in the optimal milk output of 850 ml, the diet should supply 1.15 mg of retinol, 80 mg of ascorbate, 1.5 mg of thiamin, 1.6 mg of riboflavin, 19 mg of nicotinamide, 2.5 mg of vitamin B₆, 150 µg of folate and 1.5 µg of vitamin B₁₂ every day.

A prescribed diet for lactating women is included in Table. 15.4. The diet should include adequate amounts of milk for supplying calcium, vitamin A, riboflavin, proteins and fluid, additional amounts of unsaturated vegetable oils for essential fatty acids, and animal proteins like eggs and flesh for supplying proteins of high biological value, cobalamin and other B vitamins.

15.16 DIET FOR AGED PEOPLE

Calorie requirement: Above 50 years of age, the calorie need declines progressively with age, because (a) the BMR falls on average by 7.5% in each 10 years in both sexes due to a decline in the cell population and a reduced secretion of hormones, testosterone in particular, (b) physical activities are progressively lessened particularly with retirement from occupational work, and (c) tissue growth and development cease. Thus, the total calorie requirement may decrease from 2800 kcal at 35 years of age to 2550 kcal at 55 years and 2000 kcal at 75 years in a moderately active man, and from 2200 kcal at 35 to 2000 and 1550 kcal at 55 and 75 respectively in a woman. The dietary calories should be adjusted to the declining physical activities.

Protein requirement: In spite of the decline in total calorie requirement, protein allowance should be maintained at the normal adult level of 1 g per kg of body weight per day in order to provide for the replacement of decaying tissues and also to compensate for the catabolic loss of tissue proteins in consequence of the fall in sex hormone secretion. In view of the decline in digestion and absorption, about $\frac{1}{4}$ th of the total protein should be supplied in the form of easily digestible animal proteins, having higher NPUs and biological values and containing richer amounts of B vitamins like cobalamins and thiamin. Milk is an ideal food for the aged in this respect.

Fat requirement: A reduction of 15-20 g in the fat content of the daily diet is advisable in the old age, because of (a) the decline in total calorie requirement, (b) a progressive loss of the ability to digest and absorb fat, and (c) the threat of hypercholesterolemia and hyperlipemia. The food fat should account for 15% or less of the total calories. Saturated fats should be particularly restricted and unsaturated vegetable oils should be preferred because of the higher digestibility, tocopherol

content and cholesterol-lowering effects of the latter. Supplementation with vitamins A and D may become necessary due to the restriction in the intake of saturated animal fats containing them.

Carbohydrate requirement: Reduction in carbohydrate allowance should be proportionate to the decline in calorie requirement. Fibrous polysaccharides are particularly needed to facilitate peristalsis and bowel movements. High-extraction wheat flours, richer in vitamins, minerals and fibres, are preferable to low-extraction flours, potato and other tubers.

Vitamin and mineral requirements: While the requirements of minerals and most of the vitamins do not decline with age, their intakes may become insufficient due to a fall in total food intake. Even the intakes of thiamin, riboflavin and nicotinamide, whose requirements are lessened with the decline in calorie intake, may become insufficient in the old age. Besides, the absorption of some of the micro-nutrients like iron, calcium and fat-soluble vitamins may decline in the aged. Thus, senile osteoporosis and osteomalacia may result from low intakes of Ca and vitamin D—the condition deteriorates due to reduced cutaneous synthesis of vitamin D in infirm aged people unable to move to outdoor sunshine. Nutritional anemias, either microcytic or megaloblastic, often appear and poor hemoglobin levels and low plasma folate concentrations are frequently seen. Sub-clinical forms of scurvy, indicated by low blood and tissue ascorbate levels and delay in wound healing, are relatively frequent among the aged. Poor night vision and abnormal electroretinogram may result from vitamin A deficiency. To prevent these, 100 IU of vitamin D should be taken daily in the form of fish liver oils; milk and carrots should be adequately taken to supply calcium and vitamin A. Leafy vegetables should be enhanced to supply folic acid, riboflavin and

carotenes. One fresh citrus fruit should be included in the daily food for vitamin C. Liver and lean flesh proteins should be taken to supply cobalamins, thiamin and other B vitamins. Adequate amounts of iron should be provided either as ferrous sulfate preparations or by liver, lentil and other iron-rich foods.

15.17 DIET FOR CHILDREN

Calorie requirement: In infancy and childhood, the daily calorie requirement is much higher per kg of body weight than in adults, due to (a) higher BMR in the child, (b) higher rates of tissue growth and development, (c) much higher muscular activity in play and games, and (d) the participation of older children in household work and sometimes, in employment. Thus, the energy allowance for a 9 year old child may exceed that of a sedentary adult woman (1900 kcal) by 150 kcal.

Protein requirement: Infants and children have a higher requirement for protein per kg of body weight than that of adults. The requirement declines progressively with age almost upto adolescence. Moreover, histidine is an essential amino acid for children. The requirement for essential amino acids is several times higher per kg of body weight in children than in adults. Protein malnutrition may easily produce marasmus and kwashiorkor in infants and children. Allowances should provide greater amounts of proteins per kg of body weight, particularly as milk and egg proteins having high NPU and BV. For the protein needs of about 1.8 g/kg/day in infants above 6 months in age, good quality proteins should also be provided from eggs, cereals and pulses in addition to the breastmilk. Considering the rapid deposition of proteins in the growing tissues, a daily obligatory loss of 2 mg endogenous N per kg of body weight and an average NPU of 65 for ordinary mixed Indian diets, the protein

allowance has been fixed at 1.2-1.8 g/kg/day, declining with age. About $\frac{1}{4}$ th of the total protein should be supplied as animal proteins of high NPU and BV.

Fat requirement: Infants, deprived of mother's milk, should be given so much fat in the daily food as to account for 50-60% of the total calories, resembling the situation when they are breast-fed. About 6% of their total calorie need should be supplied in the form of essential fatty acids while all polyunsaturated fatty acids, taken together, should account for a minimum of 7% of the total dietary calories. In young children, 25% of the total calories should be supplied as fat; visible fat should not exceed an amount equivalent to 15% of the total calories, the remainder being supplied as invisible fats of cereals, pulses, milk and flesh foods. Not less than 5% of the dietary calories should come from essential fatty acids.

Mineral and vitamin requirements: In view of the net total deposition of about 950 g of calcium in the bones from birth to adolescence and the continuous loss of some endogenous calcium, ICMR recommends daily calcium allowances of 0.5-0.6 g for infants and 0.4-0.7 g for children, according to age.

Throughout the period of growth, vitamin D requirement is also enhanced to help in the metabolism and retention of calcium—a daily allowance of 200 IU of vitamin D has been recommended for infants and children. Deficiency of these nutrients frequently produces rickets in Indian infants and children. Compared to body weights, requirements for vitamins A and C are also higher in infants and children because of the role of vitamin A in mucopolysaccharide formation and prevention of xerophthalmia, and that of vitamin C in collagen formation in growing tissues. About 400-750 μ g of retinol and 20-40 mg of vitamin C are recommended. Xerophthalmia is very frequent among Indian children of poorer stock. The daily loss of endogenous iron in the urine, sweat and gastrointestinal juices amounts to 5-10 μ g; about 0.2-0.5 mg iron is also deposited daily in the growing tissues as heme and non-heme irons. Considering them along with a 3% absorption of food iron in infants and children, 20-25 mg/day and 1 mg/kg/day are the ICMR-recommended iron allowances for children and infants, respectively.

A balanced diet for a child (4-6 yrs) is given in Table 15.4.

16. KIDNEYS AND LIVER

Kidneys and liver are vitally important for homeostasis, i.e., the maintenance of a constant internal environment.

16.1 NORMAL CONSTITUENTS OF URINE

Normal urine is a clear, yellowish, usually acidic (pH 4.8-8.0), aqueous liquid with an aromatic odour. Its daily volume (800-2500 ml), osmolarity (70-1200 mOsm l⁻¹) and specific gravity (1.001-1.030) depend on the intake and loss of fluid and other factors. It normally contains 93-97% water and 3-7% solids. Urinary solids include nitrogenous and non-nitrogenous organic substances, minerals, desquamated cells and small amounts of vitamins, hormones, enzymes, drugs, etc. Its normal color comes mainly from urochrome, a chromoprotein containing urobilinogen. Alkaline urine contains crystals of calcium oxalate and calcium-magnesium-ammonium triple phosphates.

16.2 FORMATION OF URINE

Urine is formed in nephrons by glomerular filtration, tubular reabsorption and tubular secretion.

Glomerular filtration

The *glomerular* or *tubular filtrate* is an ultrafiltrate filtered out from the glomerular capillary blood into the Bowman's capsule. It is filtered by an *effective filtration pressure* (P_f). When the glomerular blood pressure (P_b) averages about 60 mm Hg in humans, the osmotic pressure (P_o) of plasma proteins averages about 30 mm Hg in glomerular blood, and the pressure in the Bowman's capsule (P_c) amounts to about 18 mm Hg, $P_f = P_b - (P_o + P_c) = 60 - (30 + 18) = 12$ mm Hg.

P_c is again the sum of the pressure of the glomerular filtrate (10-12 mm Hg) collected in the Bowman's capsule and the pressure of about 6-8 mm Hg exerted on the capsule by the renal interstitial fluid and surrounding tissues.

Glomerular filtration rate (GFR) is the volume of the filtrate formed per minute by both kidneys. It may be estimated by inulin clearance and normally average 125 ml min⁻¹ in humans. *Filtration coefficient* (K_f) is the volume of one minute's glomerular filtrate per unit filtration pressure. In normal humans,

$$K_f = \frac{\text{GFR}}{P_f} = \frac{125 \text{ ml min}^{-1}}{10 \text{ mm Hg}} = 12.5 \text{ ml min}^{-1} \text{ mm}^{-1}$$

Filtration fraction is the filtered fraction of the plasma flowing per minute through functionally active tissues of both kidneys. It averages about 0.20 in normal humans.

The glomerular membrane is permeable only to particles of size ≤ 7 nm and MW < 69 kdal. Small molecules and ions such as glucose, amino acids, urea, creatinine, bilirubin, hemoglobin, Na⁺, K⁺ and Cl⁻ are filtered out, but no blood cell and very little macromolecular plasma proteins can cross the membrane. The glomerular filtrate, therefore, closely resembles the protein-free plasma in composition and normally contains only 0.02-0.03% protein. But the Donnan effect of nondiffusible plasma protein anions, occurring almost solely on the vascular side of the membrane, favours the outflow of diffusible anions into the filtrate and restricts that of diffusible cations. The filtrate consequently has higher concentrations of small anions and lower concentrations of small cations than the plasma.

TABLE 16.1. Normal constituents of human urine.

Constituent	Amount per 24 hrs 20-35 g (8-14 g N)	Source and significance
1. NPN substances		
Urea	15-32 g (6-13 g N)	Endproducts of proteins and nucleic acids. Rise due to high-protein diet, increased protein catabolism.
Creatinine	0.8-1.8 g (0.3-0.8 g N)	Chief endproduct of protein catabolism. Rise due to high-protein diet, enhanced protein catabolism, diabetes, Cushing's syndrome.
Creatine	0.06-0.5 g	Endproduct of creatine phosphate metabolism. Relatively constant. Fall in muscular dystrophy, negative N-balance, starvation.
Uric acid	0.2-0.8 g (0.05-0.2 g N)	Minor endproduct of creatine phosphate metabolism. Rise in pregnancy, hepatic carcinoma, muscular dystrophy, Grave's disease, vitamin E deficiency, starvation. Fall in myxedema.
Ammonia	0.5-1 g (0.3-0.6 g N)	Chief endproduct of purine metabolism. Rise in leukemia, gout, polycythemia, hepatitis.
Hippuric acid	0.5-0.9 g (0.04-0.08 g N)	From amide-N of glutamine. Excreted by distal tubules for base conservation. Rise in metabolic acidosis. Fall in alkalosis.
Amino acids	0.1-0.2 g N	Detoxication product of benzoic acid. Rise due to foods rich in benzoic, chlorogenic and quinic acids.
2. Other organic constituents:		
Neutral sulfate	0.07-0.12 g	Rise in De Toni-Fanconi syndrome, cystinuria, Wilson's disease, hepatic disorders, chloroform poisoning.
Ethereal sulfate	0.07-0.16 g	Product of incomplete metabolism of S-containing amino acids. Include taurine, ethyl sulfide, mercaptans, cysteine, thiocyanates, etc.
Ketone bodies	2-20 mg	Conjugation products of aromatic compounds with H_2SO_4 . Include indoxyl and skatoxyl sulfates and p-cresol sulfate.
Oxalates	15-50 mg	Products of β -oxidation. Rise in diabetes mellitus, starvation, carbohydrate deprivation.
Glucose	15-150 mg	Partly metabolic and partly dietary. Rise due to oxalate-rich foods and in primary hyperoxaluria.
3. Inorganic constituents:		
Sodium	2-4 g	Negligible normally. Rise in diabetes mellitus and renal glucosuria.
Chloride	3-4 g	Rise in Addison's disease.
Potassium	1.5-2 g	Rise in primary aldosteronism. Fall due to vomiting.
Inorganic sulfate	0.7-1 g	Rise in alkalosis, primary aldosteronism, Cushing's syndrome. Fall in acidosis, Addison's disease.
Calcium	0.08-0.25 g	Rise in increased protein catabolism.
Magnesium	0.05-0.2 g	Rise in hyperparathyroidism. Fall in parathyroid tetany.
Phosphate	1-4 g (as P_4O_{10})	Rise in hyperparathyroidism. Fall in tetany.
Iron	50-70 mg	Rise in hyperparathyroidism, vitamin D deficiency.
Iodine	50-200 μ g	Fall in tetany.
		Rise in nephrosis and proteinuria.
		Degradation product of thyroid hormones. Rise in hyperthyroidism. Fall in hypothyroidism.

Tubular reabsorption

As the glomerular filtrate flows along the tubules, (i) some solutes such as Na^+ , K^+ , glucose and amino acids are actively reabsorbed against their concentration gradients, (ii) other solutes like Cl^- and urea are reabsorbed by passive diffusion, and (iii) more than 99% of the filtered water is reabsorbed passively by osmosis (vide 16.3).

Transport maximum:

For many actively reabsorbed solutes, there exists specific maximum rates of tubular reabsorption. This rate, called the *transport maximum* (T_m) of a substance, is limited by the amounts of carrier and enzyme molecules for its transmembrane transport. This reabsorption T_m is calculated as follows:

$$T_m = (\text{amount filtered per minute}) - (\text{amount in one minute's urine}) \\ = \text{GFR} \times P - UV$$

where P and U mg ml^{-1} are the concentrations of the substance in plasma and urine respectively and V ml of urine is formed per minute. Glucose has a reabsorption T_m of about 320 mg min^{-1} .

Whenever the plasma concentration of a substance is so high that its *tubular load*, i.e., its amount in one minute's filtrate exceeds its reabsorption T_m , the filtered amount in excess of the T_m fails to be reabsorbed and consequently appears in the urine. In this way, tubular reabsorptions of glucose, phosphate, sulfate, amino acids, ketone bodies, L-ascorbate and lactate are limited by their respective T_m values (*T_m -limited reabsorption*). At low plasma concentrations, they are almost totally reabsorbed against concentration gradients.

Similarly many actively secreted substances possess specific secretion T_m values. For each such substance, secretion $T_m = UV - \text{GFR} \times P$. Para-aminohippurate has a T_m of about 68-80 mg min^{-1} . So long as its plasma concentration is sufficiently below its secretion T_m ,

the plasma can be totally cleared of PAH in a single circulation through the kidneys.

If a common carrier mediates in the T_m -limited reabsorption or secretion of a number of solutes, they compete with each other for the carrier. So, an increase in the urinary or plasma concentration of one of them, particularly of that with the highest carrier-affinity, reduces respectively the reabsorption or secretion of the others.

Renal threshold:

This is the maximum concentration of a substance in the plasma, upto which its entire filtered amount is reabsorbed and it does not appear in the urine. But whenever the plasma concentration of a substance exceeds its renal threshold, its filtered amount cannot be totally reabsorbed and the unabsorbed amount appears in the urine.

High-threshold substances such as glucose and amino acids are largely and actively reabsorbed against their concentration gradients. So, their urinary elimination is small or negligible unless their blood levels rise abnormally to exceed their renal thresholds. Glucose has a renal threshold of 170-180 mg dl^{-1} and is not normally excreted in the urine unless its blood level exceeds 180 mg .

Low-threshold substances like urea and creatinine are reabsorbed to a limited extent by diffusion. They are normally excreted in the urine in considerable amounts.

Na^+ , K^+ and Cl^- reabsorption:

About 97-99% of the filtered Na^+ and practically the entire amount of the filtered K^+ are actively reabsorbed from the tubules (Fig. 16.1).

(a) The proximal convoluted tubule reabsorbs actively about 70% of the filtered Na^+ . The luminal side of the proximal tubular epithelium is electronegative to its serosal side by about -20 mV. About one mole of O_2 is consumed by the renal tissue in actively

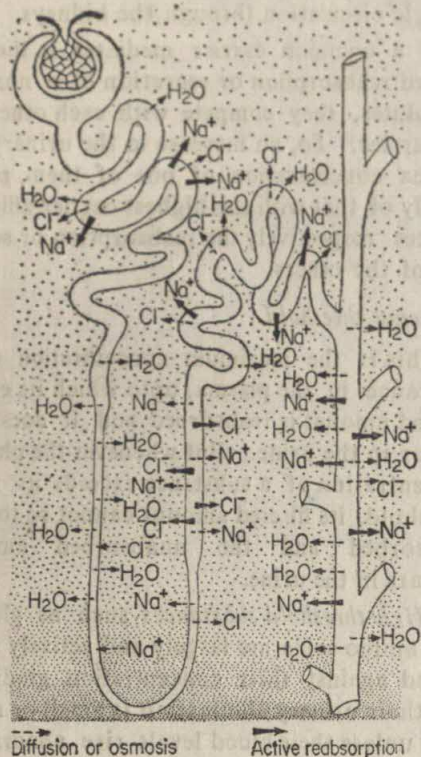


Fig. 16.1. Tubular reabsorption of Na^+ , Cl^- and water. Denser stippling indicates higher osmoconcentration.

reabsorbing 25 mEq of Na^+ against this electrical gradient as also against the concentration gradient of Na^+ . Na^+ does not show any T_m for its tubular reabsorption; so, its renal reabsorption is *not* T_m -limited. In other words, Na^+ reabsorption is not limited by any fixed number of Na^+ ions carried across the tubular membrane every minute by the membrane carrier. Instead, its reabsorption is limited by its electrochemical gradient across the tubular membrane and the period of stay of the filtrate in the tubules (*gradient-time-limited reabsorption*). The steeper the inward electrochemical gradient for Na^+ from the tubular lumen to the cell interior and the longer the stay of the filtrate in the tubules,

the higher is the tubular Na^+ reabsorption. To maintain the electrochemical gradient, Na^+ is actively transported from the tubule cell across the basal and lateral cell membranes to the intercellular channels. Cl^- simultaneously diffuses out passively through those membranes along the electrical gradient generated by the active Na^+ outflow. The Na^+ and Cl^- concentrations are thus maintained lower in the cells than in the tubular filtrate. This causes Na^+ and Cl^- to diffuse passively into the tubule cells from the tubular lumen across the luminal cell membrane (Fig. 16.2).

Because Cl^- is mostly reabsorbed by diffusion along the electrical gradient established by the active reabsorption of Na^+ , the reabsorption of Cl^- is also *gradient-time-limited* like that of Na^+ .

(b) Cl^- is actively reabsorbed from the thick ascending limb of Henle's loop, accompanied by the passive diffusion of Na^+ along the resultant electrical gradient (Fig. 16.1).

(c) Nearly $\frac{2}{3}$ ths and $\frac{1}{4}$ th of the filtered K^+ are actively reabsorbed from respectively the proximal tubule and the ascending limb of Henle's loop. K^+ reabsorption seems to be a T_m -limited process.

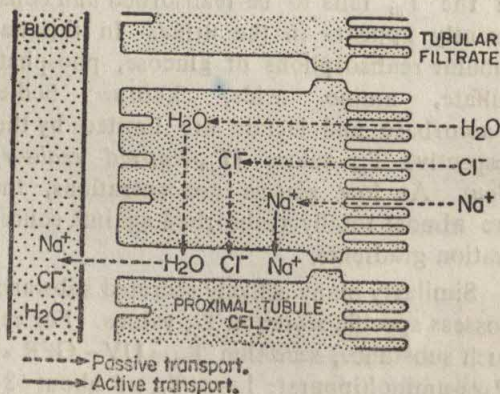


Fig. 16.2. Na^+ , Cl^- and water reabsorption in proximal tubule.

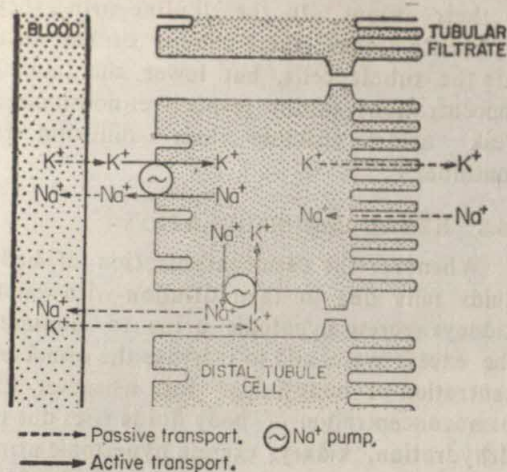


Fig. 16.3. Renal reabsorption of Na⁺ in exchange of K⁺.

(d) Na⁺ is actively reabsorbed against steeper electrochemical gradients in the distal tubule and collecting duct than in the proximal tubule. A *sodium pump* mechanism (Na⁺-K⁺-ATPase), located in the basal and lateral cell membranes of the distal tubule and collecting duct, actively carries three Na⁺ ions from inside the cell to the intercellular channels and in exchange, transports two K⁺ ions from the intercellular channel to the cell interior (Fig. 16.3). This maintains the intracellular Na⁺ and K⁺ concentrations respectively below and above their tubular concentrations and causes Na⁺ influx and K⁺ outflow by diffusion through the luminal cell membrane. Most of the urinary K⁺ is excreted by such Na⁺-K⁺ exchange in the distal tubule.

Angiotensin II and aldosterone enhance Na⁺ reabsorption from the distal tubules. PGE₂ and kinins decrease Na⁺ reabsorption to increase the urinary Na⁺ (*natriuretic effect*).

(e) Some Cl⁻ is reabsorbed by diffusion from the distal tubule and collecting duct.

Glucose reabsorption :

Glucose is actively reabsorbed in the proximal tubule by a *T_m-limited* process against

considerable concentration gradients. The *T_m* for glucose reabsorption averages about 320 mg min⁻¹ in humans while its renal threshold averages about 170-180 mg dl⁻¹. As the blood sugar level is normally lower than the renal threshold for glucose, it is totally reabsorbed and does not appear in the urine. Thyroxine increases the *T_m* to enhance glucose reabsorption. In diabetes mellitus, hyperthyroidism, acromegaly and pituitary gigantism, the blood sugar exceeds the renal threshold, causing the urinary elimination of glucose (*glucosuria*). Where *U* and *P* mg are the glucose concentrations per ml of urine and plasma respectively, and *V* ml is the volume of one minute's urine, the amount of glucose in one minute's urine is given by *UV* mg min⁻¹ : $UV = P \times GFR - T_m$.

Renal glucosuria is the urinary elimination of glucose due to a failure of its tubular reabsorption, even though the blood sugar is below the normal renal threshold. It may result from glomerulonephritis, nephrosis or a genetic deficiency of the tubular glucose carrier. Phlorrhizin administration may produce renal glucosuria by a competitive inhibition of the tubular glucose carrier system. The renal threshold for glucose is lowered below normal in renal glucosuria.

Tubular secretion

Proximal and distal tubules secrete many substances in the urine in animals having restricted water availability. Tubules of aglomerular nephrons of marine teleost fishes and adult desert amphibians like *Chiroleptes* secrete urea, creatinine, Mg²⁺, SO₄²⁻, HPO₄²⁻, Cl⁻, Ca²⁺, PAH, diodrast and phenol red. Birds, snakes, lizards and land tortoises secrete considerable amounts of uric acid in their tubules. Even a few mammals like desert rats secrete urea in their tubules.

Human proximal and distal tubules secrete the following substances.

(a) Strong organic bases such as choline, histamine, thiamin and N-methylnicotinamide are actively secreted in the proximal tubule by a *T_m-limited mechanism* using a common carrier molecule. They compete with each other for the common carrier and may inhibit each other's secretion.

(b) Strong organic acids such as PAH, hippurate, glucuronides and sulfuric acid esters as well as creatinine, penicillin and diodrast are secreted in the proximal tubule by a *T_m-limited mechanism* using another common carrier. They may also compete with each other for being secreted by the common carrier.

(c) K^+ and H^+ are actively secreted in the distal tubule and collecting duct in exchange of Na^+ which is reabsorbed by the *sodium pump*. So, like Na^+ reabsorption, secretions of K^+ and H^+ are *gradient-time-limited mechanisms*.

(d) Weak bases such as ammonia, quinine and chloroquine are excreted by passive diffusion in the distal tubule and collecting duct so long as the urine is acidic. Ammonia, for example, diffuses down its own concentration gradient into the tubular lumen across the tubular membrane which is permeable to non-ionized weak bases, but impermeable to their cations. In the acidic urine, NH_3 binds with H^+ to form NH_4^+ which cannot diffuse back into the tubule cells but lowers the tubular concentration of free NH_3 so that NH_3 continues to diffuse from the tubule cell to the tubular lumen as long as the urine is acidic. This mechanism is called *diffusion-trapping*.

(e) Weak acids such as salicylic acid and phenobarbital are also excreted passively in the distal tubule and collecting duct by the *diffusion-trapping mechanism* so long as the urine is alkaline. They diffuse from the tubule cells to the tubular lumen down their concentration gradient because the tubular membrane is permeable to them, though not

to their anions. In the alkaline urine, they ionize into anions; these cannot diffuse back into the tubule cells, but lower the tubular concentrations of the respective non-ionized weak acids, enabling their diffusion to continue.

16.3 RENAL OSMOREGULATION

Whenever the osmoconcentration of body fluids falls due to their dilution with water, kidneys secrete hypotonic urine to eliminate the excess water and to increase the osmoconcentration of body fluids. But whenever the osmoconcentration of body fluids rises due to dehydration, kidneys excrete hypertonic urine to retain water and to lower the body fluid osmoconcentration.

Formation of hypertonic urine

The glomerular filtrate is practically isotonic with the plasma and has the same osmolarity (300 mOsm l^{-1}) as the plasma. From this filtrate in the proximal tubule, many solutes like Na^+ , K^+ , Cl^- , glucose and amino acids are reabsorbed. Because the proximal tubular membrane is freely permeable to water, a proportionate volume of water is simultaneously reabsorbed by the osmotic effects of the reabsorbed solutes (*obligatory reabsorption* of water). So, the proximal tubule reabsorbs an isotonic solution of solutes and leaves an isotonic filtrate (about 300 mOsm l^{-1}) to pass into the Henle's loop (Fig. 16.4). Such reabsorption of about 65% or more of the filtered water in the proximal tubule reduces the urine volume considerably. In diabetes and Addison's disease, high tubular concentrations of respectively glucose and Na^+ reduce the obligatory reabsorption of water and consequently increase the urine volume manifold (*diuresis*).

A *counter-current exchanger* is constituted of two arms of each loop-like vasa recta vessel in the renal medullary interstitium. There is a free exchange of water as well as solutes between the blood flowing down the

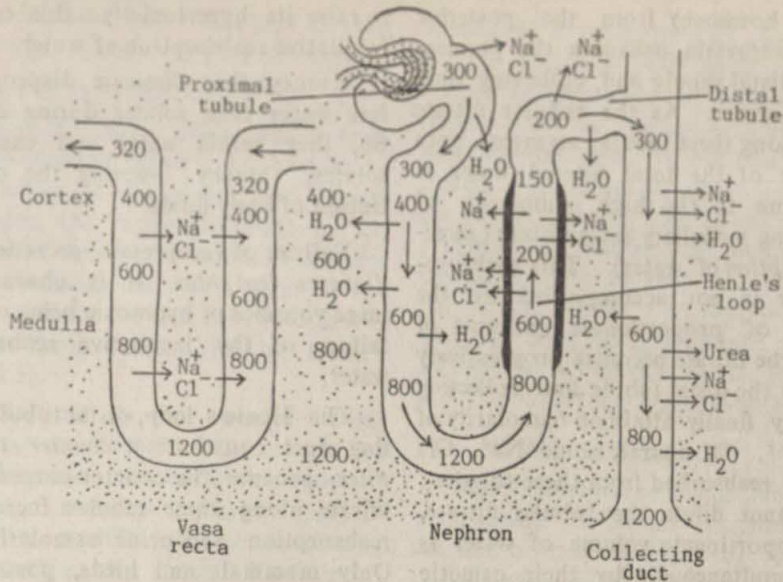


Fig 16.4. Formation of hypertonic urine. Numbers indicate osmolarity in mOsm per litre. Tubule with thick wall is impermeable to water.

descending arm of the vasa recta towards the inner medulla, and that returning along its ascending arm towards the renal cortex. This counter-current exchange retains and concentrates in the inner medulla the solutes like Na^+ Cl^- and urea reabsorbed from the ascending limb of the Henle's loop, the distal tubule and the collecting duct. This makes the medullary interstitium progressively hypertonic towards the inner medulla. When body fluids get concentrated due to dehydration, this hypertonicity of the interstitium may reach 1200 mOsm l^{-1} in the inner medulla (Fig. 16.4). The thin descending limb of Henle's loop, located in the medullary interstitium, is permeable to water but reabsorbs neither Na^+ nor Cl^- from the filtrate. As the tubular filtrate flows down the descending limb, about 15% of the total water of the glomerular filtrate gets progressively reabsorbed due to the medullary interstitial hypertonicity; besides, some Na^+ and Cl^- may diffuse into the filtrate from the more concentrated interstitial fluid. The filtrate thus becomes

progressively hypertonic and smaller in volume, attaining an osmolarity as high as 1200 mOsm l^{-1} at the tip of the loop.

The thick ascending limb of Henle's loop is always impermeable to water. So, during the return of the filtrate towards the renal cortex along the ascending limb, no water is reabsorbed from the filtrate and its volume does not change further. But some Cl^- is actively reabsorbed from the filtrate here, accompanied by some Na^+ passively reabsorbed along the electrical gradient. The filtrate consequently gets progressively diluted and hypotonic again—its osmolarity declines to about 200 mOsm l^{-1} where it enters the distal tubule.

In the distal tubule and collecting duct, Na^+ is actively reabsorbed from the filtrate, accompanied by the diffusion of Cl^- along the electrical gradient. But the membranes here are practically impermeable to water in absence of the hormone *vasopressin*. However, during dehydration, a rise in plasma osmoconcentration stimulates the secretion of *vasopressin*

(anti-diuretic hormone) from the posterior pituitary. Vasopressin enhances the permeability of the distal tubule and collecting duct membranes to water. As the tubular filtrate now flows along these tubular segments, upto 19% or more of the total filtered water is reabsorbed due to the high osmolarity of the surrounding medullary interstitium (*facultative reabsorption* of water). This reabsorption of water is not accompanied by the reabsorption of proportionate amounts of solutes; so, the filtrate becomes progressively hypertonic in the distal tubule and collecting duct and may finally attain an osmolarity of 1200 mOsm l^{-1} . Of course, some Na^+ , Cl^- and urea are reabsorbed from these tubules; but these cannot dilute the tubular filtrate, because a proportionate volume of water is reabsorbed simultaneously by their osmotic effects so long as these tubular segments remain permeable to water due to vasopressin action. Moreover, the reabsorbed ions and urea are retained in the medullary interstitium

to raise its hypertonicity; this enhances the facultative reabsorption of water.

Kidneys thus eliminate disproportionately less water than solutes during dehydration. So, they retain water and excrete excess solutes, thereby lowering the osmoconcentration of body fluids.

Failure of vasopressin secretion produces *diabetes insipidus*. It is characterized by large volumes of hypotonic urine owing to the failure of the facultative reabsorption of water.

The Henle's loop, distal tubule and collecting duct constitute a *counter-current multiplier*, because the counter-current flow of the filtrate along these tubules increases water reabsorption and urine osmolarity manifold. Only mammals and birds, possessing long Henle's loops, form hypertonic urine. Desert mammals with long-looped nephrons reabsorb much more water than semi-aquatic mammals and beavers lacking in long-looped nephrons.

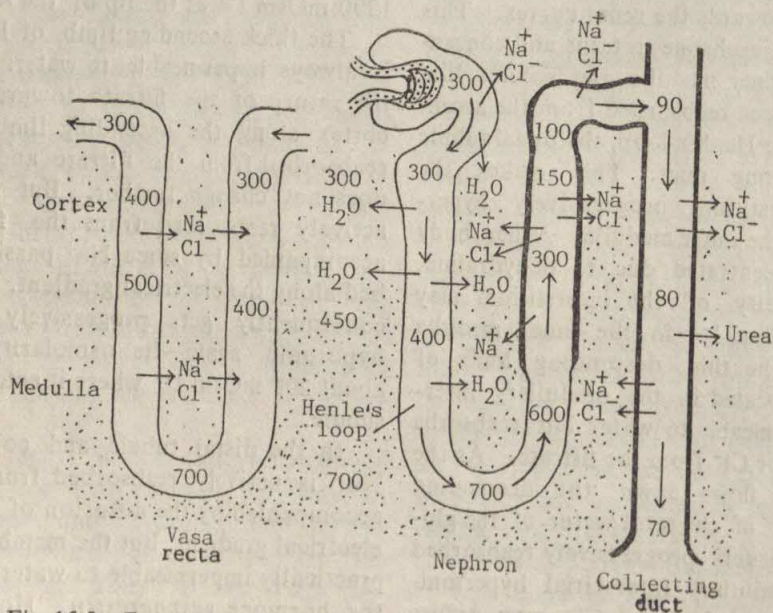


Fig. 16.5. Formation of hypotonic urine. Numbers indicate osmolarity in mOsm per litre. Tubules with thick walls are impermeable to water.

Reptiles, amphibians and fishes lack Henle's loops and cannot eliminate hypertonic urine.

Formation of hypotonic urine

Even when the body fluids suffer from osmotic dilution due to excess water, about 65% of the total water of the glomerular filtrate is reabsorbed in the proximal tubule by the osmotic effect of the solutes reabsorbed there (*obligatory reabsorption* of water). The filtrate still remains isotonic (300 mOsm l^{-1}) when it enters the descending limb of Henle's loop (Fig. 16.5).

Vasa rectae act as the counter-current exchangers to retain most of the reabsorbed Na^+ , Cl^- and urea in the medullary interstitium, making it progressively hypertonic towards the inner medulla; but its maximum osmolarity reaches only about 700 mOsm l^{-1} when a dilute urine is being formed, because far less Na^+ , Cl^- and urea are reabsorbed if the urine flows rapidly due to its large volume.

The descending limb of Henle's loop is permeable to water, but does not reabsorb Na^+ or Cl^- . As the isotonic filtrate flows down the thin descending limb, about 15% of the total filtered water is reabsorbed by the osmotic effect of the hypertonic medullary interstitium. This progressively concentrates the filtrate upto 700 mOsm l^{-1} at the tip of the loop.

No water is reabsorbed from the filtrate as it returns to the renal cortex along the ascending limb. But the filtrate gets progressively diluter and hypotonic without any change in volume, because some Cl^- and Na^+ are reabsorbed from it in the ascending limb. Thus, the osmolarity of the filtrate falls to about 100 mOsm l^{-1} where it enters the distal tubule.

As the filtrate flows down the distal tubule and collecting duct, some more Na^+ is actively reabsorbed from it, some Cl^- is reabsorbed by diffusion along the electrical gradient produced by Na^+ reabsorption, and

some urea is reabsorbed by passive diffusion. But no water is reabsorbed now from these tubular segments because their membranes are impermeable to water in absence of vasopressin—the latter is not secreted when the plasma osmoconcentration is low. Thus, the filtrate remains large in volume and gets progressively diluted and hypotonic until its osmolarity falls to only about 70 mOsm l^{-1} . So, *no facultative reabsorption* takes place when the body fluids are dilute. In this way, kidneys get rid of excess water from the body fluids to raise their osmoconcentration.

16.4 RENAL CLEARANCES

Renal clearance of a substance is the minimum volume of plasma containing the same amount of the substance as is excreted in one minute's urine.

$$C = \frac{\text{mg of the substance excreted per minute}}{\text{mg of the substance per ml of plasma}} = \frac{UV}{P}$$

where C is the renal clearance in ml min^{-1} , V ml the volume of one minute's urine, and U and P mg of the substance occur in 1 ml of respectively urine and plasma.

Renal clearance is used to estimate (i) the rate of urinary elimination of a substance, (ii) the capacity of the kidney to reabsorb or secrete it, (iii) the renal blood flow, (iv) the glomerular filtration rate, (v) the filtration fraction, and (vi) the mass of functional renal tissue.

Measurement of glomerular filtration rate

The glomerular filtration rate (GFR) is given by the clearance of any substance, excreted solely by glomerular filtration and neither reabsorbed nor excreted by renal tubules. Such substances include inulin, sodium thiosulfate, polyvinylpyrrolidone and mannitol.

Inulin clearance :

Inulin, a polyfructosan ($\text{MW} \approx 5.5 \text{ kdal}$), is neither normally present nor metabolized

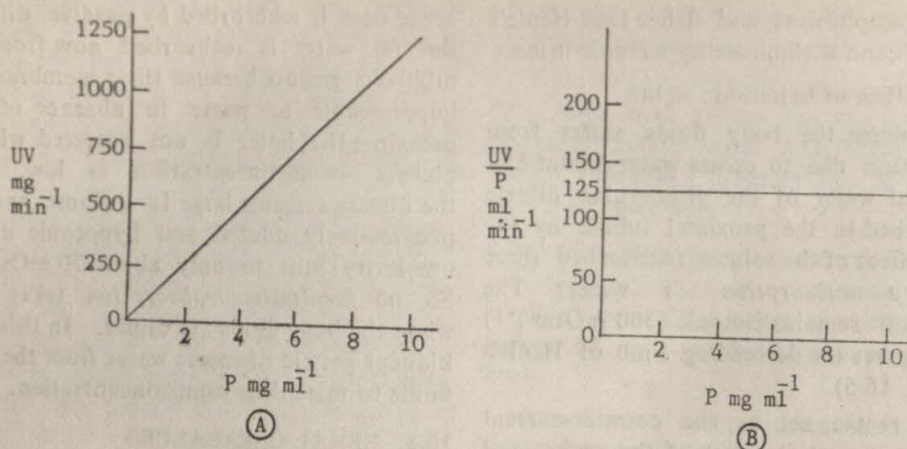


Fig. 16.6. (A) Relation between the excretion rate UV and the plasma concentration P of inulin. (B) Relation between the clearance UV/P and the plasma concentration P of inulin.

in the body; it can be intravenously injected or infused for measuring its clearance. Its small molecules easily cross the glomerular membrane. Where U mg of inulin occur in each ml of urine and V ml of urine are formed per minute, UV mg of inulin are eliminated in one minute's urine; plotting of UV against the plasma inulin concentration (P mg ml⁻¹) gives a straight line (Fig. 16.6). UV/P is thus a constant, independent of P ; so, plotting of UV/P against P gives a straight line parallel to the X axis. These indicate that inulin is freely filtered into the Bowman's capsule and neither secreted nor reabsorbed in the tubules. So, all the urinary inulin comes from glomerular filtration. Its clearance is thus a measure of GFR.

The normal inulin clearance (and so, the GFR) varies with the body surface area, sex and age. Its value averages about 125 and 110 ml/min/1.73 m² surface area in young men and women, respectively. Even per m² surface area, inulin clearance is lower in infants and old persons than in young adults.

Clearance ratio (C_x/C_i):

It is often computed to compare the clearance C_x of any substance with that of

inulin (C_i). Where U_x and U_i mg are their respective concentrations in 1 ml urine, P_x and P_i mg ml⁻¹ are their plasma concentrations, and V ml the volume of one minute's urine,

$$\frac{C_x}{C_i} = \frac{U_x V / P_x}{U_i V / P_i} = \frac{U_x P_i}{U_i P_x}$$

Clearance ratios indicate the degree of tubular reabsorption or secretion of a substance in addition to its glomerular filtration. Phenol red, para-aminohippurate (PAH), N-methylnicotinamide, diodrast and ethereal sulfates possess clearance ratios above 1.00. This indicates their tubular secretion in addition to filtration. Glucose, amino acids, L-ascorbate, Na⁺, Cl⁻, Ca²⁺, HPO₄²⁻ and urea have clearance ratios below 1.00, because they are wholly or partly reabsorbed from the glomerular filtrate in tubules.

Creatinine clearance:

Clearance of exogenous creatinine, infused intravenously, is alternatively used in measuring the GFR, because creatinine is freely filtered, but neither metabolized nor reabsorbed by the kidney. However, renal tubules secrete some creatinine to raise the UV for

creatinine; but the method for plasma creatinine estimation is not absolutely specific and overestimates the true plasma creatinine concentration. These two errors counteract, bringing the clearance ratio of exogenous creatinine down to 1.2 and consequently making its clearance a reasonable estimate for the GFR.

Endogenous creatinine, produced from muscle creatine phosphate, has a lower clearance than the GFR; its clearance is consequently not a measure of the GFR.

EDTA clearance:

Ethylene diaminetetraacetate (EDTA), labelled with gamma-emitter ^{51}Cr , is stable, metabolically inert, rapidly eliminated by glomerular filtration, and little reabsorbed or secreted by tubules. The GFR is often estimated from the renal clearance of intravenously infused ^{51}Cr -EDTA, utilizing the gamma emission of its radioisotope.

Measurement of renal plasma flow

Of the total volume of blood flowing through the kidneys, some supplies the renal capsule, perirenal adipose tissue and the renal medulla, and contributes little to urine excretion. The volume of plasma, flowing through the renal cortex, makes more significant contribution to renal excretion and is called the *effective renal plasma flow* (ERPF). The ERPF through both kidneys is estimated by the clearance of PAH, diodrast or thio-sulfate, because at low plasma concentrations ($1-2 \text{ mg dl}^{-1}$), they are totally cleared from the plasma in a single flow of the blood through the renal circulation.

PAH clearance:

PAH has a normal clearance of about 630 ml min^{-1} and a clearance ratio of about 5-6, because besides its glomerular filtration, it is also actively secreted by proximal tubules through a T_m -limited secretory process. The secretion T_m for PAH ($68-80 \text{ mg min}^{-1}$) is

attained at a plasma PAH concentration of about 10 mg dl^{-1} . Below this plasma concentration, PAH is almost totally removed from the plasma in a single flow through the kidneys, reducing its renal venous concentration practically to zero.

Fick principle for estimating the pulmonary blood flow (PBF) or cardiac minute volume is extended to the measurement of the renal plasma flow (RPF).

$$\text{PBF} = \frac{\text{O}_2 \text{ uptake per minute}}{\text{Arteriovenous O}_2 \text{ difference}}$$

$$\text{RPF} = \frac{\text{PAH excreted per minute}}{\text{Arteriovenous PAH difference}} = \frac{U_p V}{P_a - P_v}$$

where $U_p \text{ mg ml}^{-1}$ is the urinary PAH concentration, $V \text{ ml}$ of urine are excreted per minute, and P_a and $P_v \text{ mg ml}^{-1}$ are the PAH concentrations in arterial and venous bloods. When P_a is far less than the T_m for PAH, P_v amounts practically to zero.

$$\text{RPF} = \frac{U_p V}{P_a - 0} = \frac{U_p V}{P_a} \approx \text{PAH clearance}$$

Usually, P_a is estimated by measuring the PAH concentration in the plasma sample from a limb vein because such a sample has the same PAH concentration as that of the renal arterial blood.

PAH clearance does not correspond exactly to RPF, because a single circulation through the kidneys extracts only 91% and not the entire amount of PAH from the plasma. In other words, PAH has an *extraction ratio* of 91% or 0.91 during the flow of plasma through the two kidneys. PAH clearance actually gives the renal cortical blood flow which corresponds to the ERPF (see above). RPF can be computed from ERPF using the PAH extraction ratio of 0.91.

$$\text{RPF} = \frac{\text{PAH clearance}}{\text{PAH extraction ratio}} = \frac{\text{PAH clearance}}{0.91}$$

Renal blood flow (RBF) and *effective renal blood flow* (ERBF) are obtained by using the hematocrit value or packed cell volume (PCV).

$$\text{ERBF} = \frac{\text{ERPF}}{1 - \text{PCV}} = \frac{\text{PAH clearance}}{1 - \text{PCV}}$$

$$\text{RBF} = \frac{\text{PAH clearance}}{0.91 (1 - \text{PCV})}$$

ERPF and ERBF amount to about 600-700 and 1100-1250 ml min⁻¹ in normal humans.

Diodrast clearance :

Diodrast clearance is also used to measure the renal plasma flow as long as its plasma concentration is kept lower than that for its T_m of 57 mg min⁻¹. Diodrast has a lower clearance than that of PAH, viz., 575 ml min⁻¹ normally, because only about 85% of the total circulating diodrast is removed by kidneys in a single circulation, i.e., diodrast has an extraction ratio of 0.85. Thus,

$$\text{RPF} = \frac{\text{diodrast clearance}}{0.85}$$

Filtration fraction

This is the fraction of ERPF filtered into the Bowman's capsule.

$$\text{Filtration fraction} = \frac{\text{GFR}}{\text{ERPF}} = \frac{\text{Inulin clearance}}{\text{PAH clearance}}$$

With the normal inulin and PAH clearances of 125 and 630 ml min⁻¹, it amounts to 125/630 or 0.20.

Tubular excretory mass

This is the effective mass (mg) of tubular tissue participating in one minute's renal excretion. This is estimated from the clearance of PAH or diodrast after its administration in a dose too high to be totally removed from the blood even at the maximum rate of its urinary elimination. Its normal human value averages 50 and 76 mg min⁻¹ respectively for PAH and diodrast clearances.

Renal capacity for solute and water excretion

Osmolal clearance (C_o) and solute-free water clearance (C_w) are estimated for such purposes,

Osmolal clearance :

This is the volume of plasma water totally cleared of all solutes in one minute. It equals that volume of plasma which would dissolve the solutes, excreted in one minute's urine, to give an isotonic solution. It gives an estimate of the renal capacity for conserving water and eliminating hypertonic urine. It is estimated (C_o ml min⁻¹) from P_o and U_o mOsm which are the osmolarities of respectively plasma and urine measured by the freezing point depression method, and the volume V ml of one minute's urine : $C_o = U_o V / P_o$. With the urine getting progressively hypertonic, proportionately less and less water is excreted compared to the solutes and C_o rises progressively.

Solute-free water clearance :

During the elimination of hypotonic urine, the proportion of solutes of glomerular filtrate excreted in the urine is far lower than the proportion of filtrate water in the urine. *Solute-free water* is the volume of water eliminated in excess of that for eliminating the urinary solutes in an isotonic solution. Its clearance (C_w ml min⁻¹) is the volume of plasma cleared of this excess water per minute. C_w estimates the renal capacity for eliminating dilute urine so as to excrete the excess water and lower the water : solute ratio in the body fluids. C_w is given by the difference between V and C_o : $C_w = V - C_o$. Whenever hypotonic urine is eliminated, C_o falls and C_w consequently becomes positive. Elimination of hypertonic urine is accompanied by a negative C_w .

Urea clearance

It estimates the renal efficiency in excreting NPN. A low urea clearance indicates renal failure.

Urea clearance depends largely on the glomerular filtration rate, because the urinary urea comes from glomerular filtration. But

its clearance is considerably lower than the GFR—the clearance ratio of urea and inulin varies normally around 0.60, indicating that 30-40% of the filtered urea is normally reabsorbed. Moreover, urea clearance declines with the fall in the rate of urine secretion.

When urine is secreted at the high rate of 2 ml or more per minute ($V \geq 2 \text{ ml min}^{-1}$), the glomerular filtrate passes so rapidly along the tubules that a constant low quantity of urea, averaging normally about $\frac{2}{3}$ th of the filtered urea, is reabsorbed by diffusion. The urea clearance is then maximum and amounts to about $\frac{2}{3}$ th of the GFR. Thus, as long as $V \geq 2 \text{ ml min}^{-1}$ in an average normal human, the urea clearance amounts to about 75 ml min^{-1} and is called the *average normal maximum urea clearance*. It corresponds to a clearance ratio of about 0.60. Hence, the urea clearance (UV/P), measured in a patient secreting urine at the rate of 2 ml or more per minute, is expressed as his *maximum urea clearance*; this gives the observed urea clearance as the percentage of the average normal maximum urea clearance.

$$\begin{aligned} \text{Maximum urea clearance (\% average normal)} \\ &= \frac{100 \times \text{observed urea clearance}}{\text{average normal maximum urea clearance}} \\ &= \frac{100 \text{ UV/P}}{75} = \frac{1.33 \text{ UV}}{P} \end{aligned}$$

When urine is secreted at a rate lower than 2 ml min^{-1} ($V < 2 \text{ ml min}^{-1}$), urea clearance falls progressively with the decline in the rate of urine flow and becomes progressively less than $\frac{2}{3}$ th of the GFR. This results from a progressive rise in tubular reabsorption of the filtered urea because of a slower movement and longer stay of the filtrate in the tubules. In such conditions, urea clearance normally varies as a function of $\sqrt{V}$. In normal humans secreting urine at the rate of 1 ml min^{-1} ($V = 1 \text{ ml min}^{-1}$), the urea clearance averages 54 ml min^{-1} and is called the *average normal standard urea clearance*. This corresponds to a clearance

ratio of less than 0.44. The urea clearance (UV/P), measured in a patient when $V < 2 \text{ ml min}^{-1}$, is expressed as his *standard urea clearance*; this gives the observed urea clearance as the percentage of the normal average for the relevant rate of urine flow—the normal average is computed by multiplying the average normal standard urea clearance by $\sqrt{V}$.

$$\begin{aligned} \text{Standard urea clearance (\% normal average)} \\ &= \frac{100 \times \text{observed urea clearance}}{\text{average normal standard urea clearance} \times \sqrt{V}} \\ &= \frac{100 \text{ UV/P}}{54 \sqrt{V}} = \frac{1.85 \text{ U} \sqrt{V}}{P} \end{aligned}$$

Urea clearances of less than 20 ml min^{-1} are usually accompanied by the onset of clinical symptoms of renal failure.

16.5 RENIN-ANGIOTENSIN SYSTEM

Renin is a proteolytic enzyme secreted into the blood of afferent glomerular arterioles by juxtaglomerular (JG) cells on their walls.

Secretion of renin :

(a) JG cells are stimulated to secrete renin when renal baroreceptors are stimulated by a *fall in the arterial blood pressure*. This may result from either a *decrease in the circulating blood volume* due to hemorrhage, venous pooling of blood or constriction of vena cava, or a *peripheral vasodilatation*.

(b) The glomerular filtration rate (GFR) may be lowered by an atherosclerotic occlusion of the renal artery, a fall in the circulating blood volume or a decline in the arterial BP. A fall in GFR decreases the urine flow and consequently enhances the gradient-time-limited reabsorption of Na^+ and Cl^- from the tubular filtrate. So, the filtrate reaching the distal tubule has low Na^+ and Cl^- concentrations. Salt depletion may also decrease Na^+ and Cl^- concentrations in the tubular filtrate. *Low Na^+ or Cl^- concentration* in the distal tubule stimulates the macula densa cells on its wall. These cells probably release a

chemical substance which stimulates the neighbouring JG cells to secrete renin.

(c) A change from supine to erect posture or a fall in the circulating blood volume stimulates *adrenergic sympathetic neurons*. Noradrenaline, released at their terminals near the JG cells, binds with β -adrenergic receptors on the JG cell membrane to stimulate those cells for renin secretion.

In supine humans, the plasma renin activity is $1.3 \text{ ng ml}^{-1} \text{ hr}^{-1}$ with normal Na^+ intake; but it rises to $3.6 \text{ ng ml}^{-1} \text{ hr}^{-1}$ in salt depletion or in the standing posture.

(d) *Prostaglandins E*, *prostacyclins*, *bradykinin* and *glucagon* stimulate the release of renin from JG cells.

(e) A fall in blood K^+ or intracellular Ca^{2+} may stimulate renin secretion. The secretion is reduced in reverse situations.

Formation of angiotensins:

Renin hydrolyzes the peptide bond between leucine residues at the 10th and 11th positions from the N-terminal end in *angiotensinogen* (renin substrate), a plasma α_2 -globulin, to produce a decapeptide called *angiotensin I* and a polypeptide of >400 amino acids (Fig. 16.7). This seems to be the rate-limiting step in angiotensin synthesis. Cortisol and estradiol enhance this reaction by increasing the hepatic synthesis of angiotensinogen. Renin continues circulating and hydrolyzing angioten-

sinogen for about an hour. Renin is inhibited by a plasma phosphatidylserine. Renin secretion is inhibited by the feed-back effect of angiotensin II.

A Ca^{2+} -dependent glycoprotein called *angiotensin I-converting protein* occurs on the walls of small vessels of lungs and in the plasma. It rapidly hydrolyzes the peptide bond between phenylalanine and histidine residues at the 8th and 9th positions, respectively, in angiotensin I. This cleaves the latter into an active octapeptide called *angiotensin II* and an inactive dipeptide histidyl-leucine. Angiotensin II is the primary hormone of the renin-angiotensin system. Small amounts of it may be hydrolyzed into the active heptapeptide *angiotensin III* by *aspartate aminopeptidase* which splits off the N-terminal aspartate. Both angiotensins II and III circulate and act for minutes only and are rapidly hydrolyzed by *angiotensinases* of plasma, kidneys and intestines.

Actions of renin-angiotensin system:

Angiotensins II and III, formed by renin action, help to restore a falling ECF volume and a declining arterial BP. Angiotensin II is quantitatively more effective in humans.

(a) Angiotensin II *constricts arterioles* strongly to increase the peripheral resistance and thereby raises the arterial BP. The renin-angiotensin system requires for its activation

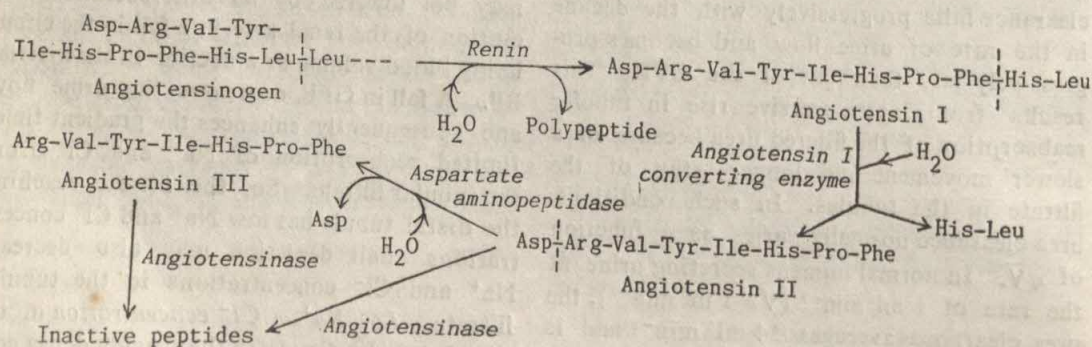


Fig. 16.7. Formation and breakdown of angiotensins.

a continuation of arterial hypotension for more than 5-10 minutes. So, this system takes longer than the sympathetico-adrenal system in exerting its full vasoconstrictor effect, and also continues to act for a longer period. After a hemorrhagic decline in the mean arterial pressure by 50%, the renin-angiotensin system can restore the BP halfway back to normal in 20 minutes. PGE_2 opposes the vasoconstrictor action of angiotensin II.

(b) Angiotensin II *constricts veins moderately*. This decreases the vascular volume and compliance, considerably increases the mean circulatory filling pressure and the venous return to the heart, and consequently enables the heart to pump blood with a greater force against the enhanced BP.

(c) Angiotensin II constricts mainly the efferent glomerular arterioles in the kidneys. This *efferent arteriolar vasoconstriction* decreases the renal blood flow, but increases the glomerular blood pressure and consequently the GFR. In prolonged hypotension, this mechanism maintains the GFR for hours. Simultaneously, the decrease in the renal blood flow spares some blood for elevating the arterial BP in the external field.

(d) By raising the GFR, the renin-angiotensin system maintains an almost *normal urea clearance* during arterial hypotension.

(e) The renin-angiotensin system may directly help in the *renal retention of water and salts* during arterial hypotension.

(i) By reducing the renal blood flow, angiotensin II reduces the peritubular capillary pressure and consequently enhances the tubular reabsorption of water and minerals.
(ii) It may directly increase the active reabsorption of Na^+ in the distal tubules.
(iii) A larger proportion of the plasma fluid is filtered into the Bowman's capsule due to slower blood flow in glomeruli. This enhances the osmotic pressure of plasma

proteins in the peritubular capillary blood and consequently increases the osmotic reabsorption of water from the distal tubules and collecting ducts.

(f) The renin-angiotensin system stimulates *aldosterone secretion*. Angiotensin II binds with specific membrane receptors on adrenal zona glomerulosa cells and thereby enhances cytoplasmic concentrations of Ca^{2+} and inositol 1,4,5-triphosphate. These second messengers in turn enhance the conversion of cholesterol to pregnenolone and that of corticosterone to aldosterone. Aldosterone thus secreted increases the active tubular reabsorption of Na^+ and consequently, the passive reabsorptions of Cl^- and water.

Through the actions described in (e) and (f), the renin-angiotensin system increases the renal retention of water, thereby *restores the falling ECF volume* and consequently helps in the *long-term increase of arterial BP*.

(g) Angiotensin II stimulates the *synthesis and release of prostaglandins* in the renal medulla. PGE_2 opposes the action of angiotensin on Na^+ and water retentions.

16.6 KALLIKREIN-KININ SYSTEM

Kallikreins are peptidases occurring as inactive pre-kallikreins in the blood, tissue fluids and glandular tissues. Pre-kallikreins are activated to kallikreins when blood is diluted or comes in contact with foreign surfaces or gets its cells damaged. Kallikreins are inactivated by a kallikrein-inhibitor of body fluids.

Kininogens are α_2 -globulins of plasma and tissue fluids. Kallikreins hydrolyze kininogens to produce the nonapeptide *bradykinin* and the decapeptide *kallidin* (lysylbradykinin) in plasma and tissues (Fig. 16.8). Kallidin can in turn be hydrolyzed by a plasma aminopeptidase into bradykinin and lysine. Prostaglandins enhance the kinin formation. Kinins are inactivated in a few minutes by

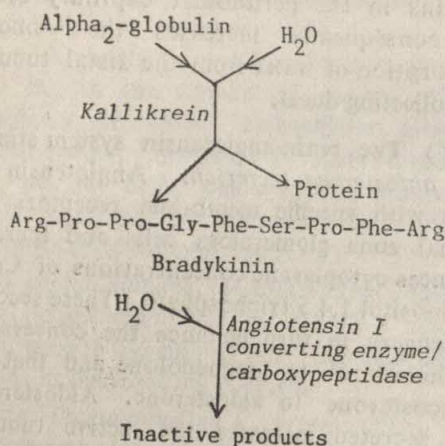


Fig. 16.8. Formation and breakdown of bradykinin.

plasma and tissue kininases and a plasma carboxypeptidase.

Distal tubule cells secrete kallikrein into the tubular lumen. Kallikrein hydrolyzes kininogens of the tubular filtrate into kinins. Kinins are, therefore, in considerable concentration in the tubular filtrate in distal tubules and collecting ducts. Some biological actions of kinins are as follows.

(a) Kinins are powerful *vasodilators*. Their vasodilator action may lower the blood pressure and enhance the blood flow in tissues such as kidneys, skin and gastrointestinal system. Through renal vasodilatation, kinins may manifold increase the blood flow in the renal medulla and inner cortex. The blood flow in the brachial artery is increased sixfold on injection of only $1\mu\text{g}$ of bradykinin into it.

(b) Kinins increase *capillary permeability*.

(c) When produced by enzymes from damaged cells, kinins cause enhanced blood flow and extravasation of fluids from the capillaries in inflamed tissues by their vasodilator and permeability-enhancing actions. They thus help in producing *inflammations*.

(d) Kinins may *stimulate nociceptors* in inflamed tissues to produce pain.

(e) The presence of kinins in the distal tubular filtrate stimulates prostaglandin synthase activity to enhance PGE_2 synthesis in distal tubules and collecting ducts. PGE_2 , in turn, may mediate in the renal effects of kinins. But in case of sodium depletion or a reduced ECF volume, kinins may modulate the renal effects of prostaglandins by enhancing the conversion of PGE_2 to $\text{PGF}_{2\alpha}$ having little renal effect.

(f) Kinins reduce *urinary osmolarity* and produce *diuretic* and *natriuretic* (Na^+ -eliminating) effects. These are mediated partly through the renal release of PGE_2 .

16.7 ERYTHROPOIETIN

Erythropoietin is a circulating acidic glycoprotein. Its oligosaccharide moiety is rich in sialic acids and hexosamines. Podocytes of the Bowman's capsule membrane probably release an enzymatic factor called *erythrogenin* in the glomerular blood. Erythrogenin probably cleaves a large circulating globulin into erythropoietin in glomerular capillaries.

Erythropoietin promotes erythropoiesis in the red bone marrow, in response to either hemorrhage or low arterial oxygen tension (Po_2). It stimulates the mitosis and differentiation of erythropoietic stem cells like proerythroblasts in the red marrow to increase the population of cells capable of hemoglobin synthesis.

Low arterial Po_2 and β -adrenergic effects of sympathetic nerves are the principal factors in stimulating erythropoietin formation. Section of splanchnics or use of beta-blockers decreases the rise in erythropoietin activity in response to low Po_2 . Renal failure or bilateral nephrectomy reduces the plasma erythropoietin activity, erythrocyte count and hemoglobin concentration. Polycystic kidney, renal carcinoma, renal ischemia, nutritional anemia or hypoxia enhances the erythropoietin activity and raises the erythrocyte count.

16.8 ABNORMAL CONSTITUENTS OF URINE

Glucose :

Glucose appears in the urine (*glucosuria*) either when the blood sugar exceeds its renal threshold in diabetes mellitus, hyperthyroidism, Cushing's syndrome, acromegaly and gigantism, or when its renal threshold is lowered (*renal glucosuria*) in glomerulonephritis, nephrosis and a genetic deficiency of the renal glucose transporter.

Ketone bodies :

These appear in the urine (*ketonuria*) in ketosis due to starvation, diabetes mellitus, hepatic glycogenoses and thyrotoxicosis.

Proteins :

Albumins form the major urinary proteins in most abnormal states (*albuminuria*).

(a) *Functional albuminuria* may sometimes occur on prolonged standing (*orthostatic proteinuria*), or in the last trimester of pregnancy. (b) *Renal albuminuria* results from pathological changes in the glomerular membranes in glomerulonephritis, hypertensive lesions of kidneys and renal infections. (c) *Post-renal albuminuria* results from lesions in the bladder, urethra, renal pelvis and prostate. (d) *Pre-renal albuminuria* results from secondary renal affectations in fever, cardiac failure, dehydration and high abdominal pressure.

Creatine :

Creatinuria or abnormally high urinary creatine occurs mainly in muscular dystrophy and also in hyperthyroidism, carbohydrate deprivation and diabetes mellitus.

Bile pigments :

(a) In *obstructive jaundice*, conjugated bilirubin (bilirubin glucuronide) increases in the urine due to its reabsorption into blood from obstructed bile ducts.

(b) In *hepatic cirrhosis*, infective hepatitis and hepatic necrosis, unconjugated bilirubin

appears in the urine due to the hepatic failure in removing bilirubin from blood, and in conjugating and excreting it in the bile.

(c) In *hemolytic jaundice* following malaria, hemolytic anemia and noncompatible blood transfusions, both conjugated and unconjugated bilirubins rise in the urine due to the hepatic failure in removing and excreting large excesses of plasma bilirubin.

(d) In *Crigler-Najjar syndrome*, unconjugated bilirubin rises in the urine due to a genetic deficiency of bilirubin-UDP glucuronyl transferase, the enzyme for conjugating bilirubin with glucuronate.

Blood :

Hematuria is the urinary appearance of blood with intact erythrocytes; e.g., in type I glomerulonephritis, malignancies, acute infections and bleedings in the urinary tract.

Hemoglobin :

Hemoglobinuria is the urinary appearance of free hemoglobin without intact erythrocytes; this may happen when free hemoglobin exceeds its renal threshold of 150 mg dl^{-1} in the blood; e.g., in malaria, blackwater fever, hemolytic jaundice and incompatible blood transfusions.

16.9 LIVER FUNCTION TESTS

Tests for carbohydrate metabolism

Galactose tolerance tests : These are based on the capacity of the liver to change galactose to glucose or glycogen. The fasting patient is administered orally or intravenously a measured amount of galactose in solution and his blood galactose is estimated at regular intervals upto 2 hours. An abnormally high and prolonged rise in blood galactose, as happens in hepatic cirrhosis and infective hepatitis, is interpreted as a failure of the liver to remove galactose from the blood for metabolism.

Glycogen storage test : This is based on the capacity of the liver for glycogenolysis in response to adrenaline. The blood sugar is estimated in the fasting patient and also at repeated intervals in the first hour after adrenaline injection. Failure of the blood sugar to rise by at least 40 mg indicates either a hepatic dysfunction or an inborn error of glycogenolysis.

Hippuric acid test for detoxication

The capacity of the liver to detoxicate benzoate into hippurate is assessed by estimating the latter in one hour's urine after an intravenous injection of 1.77 g of sodium benzoate dissolved in 20 ml water; hepatic damage, due to acute or chronic liver disease, is indicated if less than 0.7 g of hippuric acid is excreted in the first hour's urine.

Tests for excretory functions

Bromosulphthalein test : This assesses the capacity of the liver to excrete bromsulphthalein. Serum bromsulphthalein is estimated in the fasting patient 25 and 45 minutes after an intravenous injection of a 5% solution of 5 mg bromsulphthalein per kg of body weight. Hepatic removal of the dye from blood is slowed in diffuse and extensive liver damages like chronic hepatitis and hepatic cirrhosis, retaining more than 15% and 5% of the dye in the serum after 25 and 45 minutes respectively.

Bilirubin tolerance test : Bilirubin is estimated in serum samples collected before as also 5 minutes and 4 hours after an intravenous injection of 1 mg of bilirubin per kg of body weight. The percentage of retained bilirubin exceeds 5% in hepatic dysfunctions.

Tests for bile pigment metabolism

Van den Bergh reactions : (a) *Direct reaction* : To estimate only the conjugated bilirubin ('direct-reacting' bilirubin), the serum is mixed with aqueous diazo reagent containing diazotized sulfanilic acid; only water-soluble bilirubin glucuronides react readily with the reagent in an aqueous medium

to form reddish purple azobilirubin which is estimated photometrically with reference to a methyl red standard. The value exceeds 0.25 mg dl^{-1} in obstructive jaundice. (b) *Indirect reaction* : In this case, the serum is mixed with both methanol and the reagent; both free and conjugated bilirubins react with the reagent in methanol medium to produce azobilirubin whose color is estimated photometrically. The difference between the values of this total bilirubin and the 'direct-reacting' bilirubin gives the value of water-insoluble, free or 'indirect-reacting' bilirubin. In hemolytic jaundice, 'indirect-reacting' bilirubin exceeds 1.25 mg dl^{-1} .

Icteric index : The intensity of yellow color of the serum due to bilirubin may be estimated photometrically with reference to a standard potassium dichromate solution to assess the degree of jaundice. The patient's serum is diluted with 0.9% NaCl solution until it approximately matches a standard 0.1% solution of potassium dichromate in color; the intensities of the colors of the two solutions are then compared photometrically.

Icteric index

$$= \frac{10 \times \text{Reading of unknown} \times \text{Dilution factor}}{\text{Reading of standard}}$$

An icteric index exceeding 6 indicates hyperbilirubinemia with either subclinical or clinical jaundice.

Tests for protein metabolism

Thymol turbidity test : The turbidity, produced in the serum sample on adding a thymol solution in barbitone buffer, is estimated photometrically. The normal range of 0-3 MacLagan units per dl of serum is exceeded when mainly γ -globulins rise abnormally in blood, e.g., in viral hepatitis, primary biliary cirrhosis, multiple myeloma and lymphogranuloma.

Zinc sulfate turbidity test : Turbidity, produced in the serum sample on adding a ZnSO_4 solution in barbitone buffer, is

estimated photometrically. The normal range of 2-8 units is exceeded when γ -globulins rise abnormally in blood, e.g., in infective hepatitis.

Cephalin cholesterol flocculation test: An aqueous emulsion of cephalin and cholesterol is added to a serum sample diluted with 0.9% NaCl and the mixture is kept standing for 24-48 hours. An abnormal rise in serum globulins, particularly γ -globulins, produces flocculation in the mixture. A positive test is obtained in viral hepatitis even before the manifestation of jaundice; obstructive jaundice without active hepatic damage produces negative results.

Serum cholesterol test

The ratio of cholesterol and cholesteryl esters is estimated in a serum sample. In viral hepatitis and other intrahepatic jaundices, esterified cholesterol is relatively lowered while obstructive jaundice may not produce any change in the ratio.

Serum enzyme activities

Activities of glutamate-pyruvate transaminase, glutamate-oxaloacetate transaminase, alkaline phosphatase and pseudocholinesterase are estimated in the patient's serum for liver function tests (see pages 111-112).

17. MEMBRANE AND STRUCTURAL PROTEINS

Each cell is enclosed by a plasma membrane. Eukaryotic cells also possess intracellular membranes enclosing organelles like the nucleus and mitochondria. Lipids and proteins are essential constituents of all membranes. Filaments and microtubules made of proteins form the structural framework or cytoskeleton in the cytoplasm of the cell. Proteins are important constituents of cell junctions connecting adjacent cells. Collagens are extracellular structural proteins forming connective tissue fibres. Contractility of muscle fibres depends on their structural proteins, actin and myosin.

17.1 MEMBRANES

The plasma membrane is a thin, continuous, film-like, quasifluid membrane of 6-9 nm thickness. Its lipid-protein ratio ranges from 1 : 0.85 to 1 : 1.5. Proteins, lipids and carbohydrates constitute about 52%, 40% and 8% of the erythrocyte membrane. Organelle membranes possess similar structures.

Membrane models

Gorter-Grendel lipid bilayer model :

Gorter and Grendel (1925) proposed that the erythrocyte membrane is composed of a lipid bilayer consisting of two monolayers of amphipathic lipid molecules. The molecules of each lipid monolayer have their polar head-groups located on the corresponding water-adjointing outer surface of the membrane while their nonpolar hydrocarbon tails are oriented into the middle core of the bilayer (Fig. 17.1). This bilayer model satisfies the thermodynamic

stability of the membrane by providing the minimum energy configuration of amphipathic molecules in water. Thickness, refractive index, capacitance and dielectric constant of natural membranes are also closely similar to those of artificial lipid bilayers. But this model cannot explain why natural membranes differ widely from artificial lipid bilayers in resistivity, water permeability, surface tension and membrane potential.

Davson-Danielli trilaminar model :

H. Davson and J. Danielli (1935) proposed a trilaminar model of the membrane to explain its observed resistivity and low interfacial tension. They proposed that the plasma membrane was a symmetrical three-layered structure having a middle lipid bilayer bounded on either side by a continuous layer of globular polar proteins (Fig. 17.2). The globular proteins of each outer layer were supposed to be electrostatically held by the charged polar head-groups of the adjoining lipid monolayer of the middle bilayer. But such surface layers of globular proteins could not satisfactorily explain the high membrane impedance. Modi-

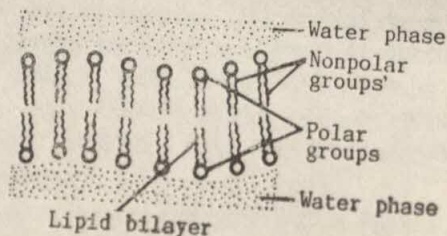


Fig. 17.1. The lipid bilayer model.

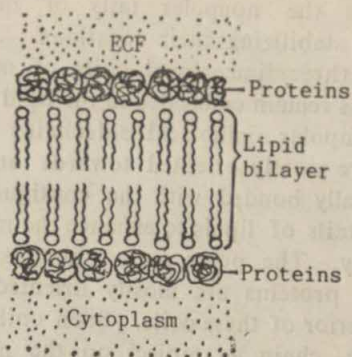


Fig. 17.2. The trilaminar model of membrane.

fied versions suggested α -helical and β -pleated forms of surface proteins instead of their globular conformations. A revised version proposed many "fixed" hydrophilic pores (diameter ≈ 0.7 nm) across the lipid bilayer. Such pores, lined by the polar sidechains of proteins, would help in the transmembrane fluxes of polar solutes like K^+ and Cl^- .

Unit membrane :

Electron microscopy subsequently revealed a trilaminar appearance of the plasma membrane and organelle membranes in osmium/permanaganate-fixed tissues. So, J. D. Robertson

(1959) proposed a common trilaminar structure for all membranes. He called it a *unit membrane* and interpreted each of its two electron-dense outer layers as a surface layer of proteins, and the electron-lucent middle layer as the layer of nonpolar fatty acid tails of a lipid bilayer intervening between the electron-dense layers. Stoecknius, however, demonstrated a similar trilaminar appearance of osmium-fixed artificial lipid bilayers free from proteins. This indicates that the outward directed polar head-groups of the lipid bilayer also contribute to the electron-dense layers. Moreover, cellular membranes retain their trilaminar appearance even after extraction of their lipids.

Singer-Nicolson fluid mosaic model :

Electron micrographs of freeze-fracture replicas of the plasma membrane show that the fracture face, passing along the midline between the lipid monolayers, bristles with many particles of 8.5 nm diameter. Such particles evidently remain deeply embedded in the lipid bilayer and extend across the fracture face. But freeze-fracture replicas of protein-free artificial lipid bilayers do not show such particles while artificial lipid bilayers,

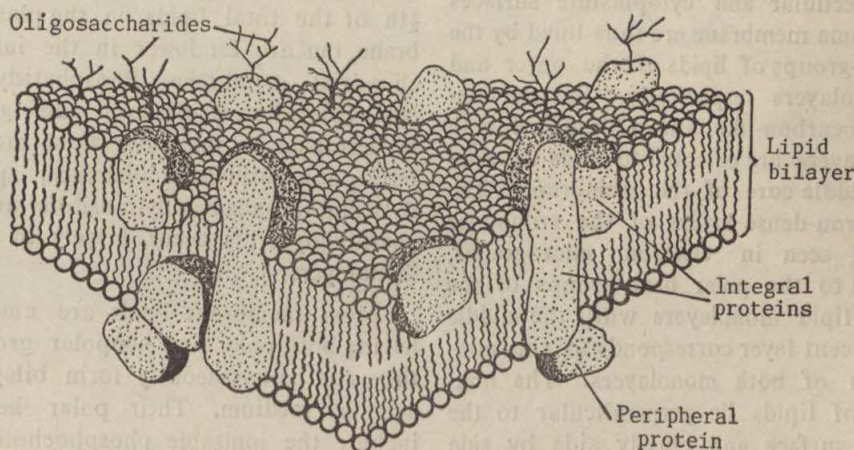


Fig. 17.3. The fluid mosaic model of membrane. [After L. C. Junqueira, J. Carneiro and J. A. Long, *Basic Histology*, 5th ed., Appleton & Lange, 1986.]

mixed with a membrane protein, show those particles on the fracture face. Addition of proteins beneath a phospholipid monolayer at a water-air interface reduces its surface tension, indicating that protein chains penetrate into the lipid monolayer. Phospholipids and proteins were also shown to redistribute themselves rapidly in the membrane, indicating its fluidity.

To explain such findings, S. J. Singer and G. L. Nicolson (1972) proposed a *fluid mosaic model* of the membrane. It describes the membrane as consisting of a continuous quasi-fluid lipid bilayer in which many globular protein molecules remain embedded as a discontinuous mosaic of particles interrupting the continuity of the lipids at scattered points (Fig. 17.3). Some of these membrane proteins extend either considerably into both monolayers or all across the lipid bilayer. These appear as the 8.5 nm particles on the fracture face of the membrane or as transmembrane bridges in high resolution electron micrographs of membranes. The lipid bilayer consists of two lipid monolayers. The polar head-groups of the amphipathic lipids of each monolayer are located on the surface of the monolayer to face the adjoining aqueous phase. The extracellular and cytoplasmic surfaces of the plasma membrane are thus lined by the polar head-groups of lipids of the outer and inner monolayers respectively. But the non-polar hydrocarbon-like tails of lipids of both the monolayers are oriented towards the non-aqueous middle core of the membrane. The outer electron-dense layers of the trilaminar membrane, seen in electron micrographs, correspond to the polar head-groups of the respective lipid monolayers while the middle electron-lucent layer corresponds to the non-polar tails of both monolayers. The non-polar tails of lipids lie perpendicular to the membrane surface and closely side by side with each other in a monolayer. This provides extensive hydrophobic interactions

between the nonpolar tails of membrane lipids, stabilizing their locations. Stretches of the three-dimensional coils of membrane proteins remain embedded in the lipid bilayer. The nonpolar amino acid sidechains of these coils are mostly oriented towards and hydrophobically bonded with the contiguous non-polar tails of lipids to enhance the membrane stability. The polar amino acid sidechains of the proteins are mostly oriented towards the interior of these coils. Such coils of the peptide chain as project on the membrane surface, bear mostly polar amino acid sidechains which associate with the adjoining aqueous phase. Lining of the membrane surfaces by the polar head-groups of lipids and the polar amino acid sidechains of proteins serves to stabilize the water-lipid interface.

Membrane lipids

Membrane lipids form about 40-50% of the membrane. Phospholipids, particularly phosphatidylcholines, phosphatidylethanolamines and sphingomyelins constitute about 50-65% of the membrane lipids; phosphatidylserines, phosphatidylinositols, phosphatidylglycerols, cerebrosides, sulfatides, gangliosides and cardiolipins form only $\frac{1}{3}$ - $\frac{1}{10}$ th of the lipids; cholesterol and its esters constitute about $\frac{1}{4}$ th of the total lipids in the plasma membrane, but are far lower in the inner mitochondrial membrane. Phosphatidylcholines, sphingomyelins, cholesterol and glycolipids predominate in the outer lipid monolayer; but phosphatidylethanolamines, phosphatidylserines, phosphatidylinositols and phosphatidylglycerols occur largely in the inner monolayer.

Most membrane lipids are amphipathic, having both polar and nonpolar groups. So, they can spontaneously form bilayers in the aqueous medium. Their polar head-groups include the ionizable phosphocholine, phosphoethanolamine and phosphoamino acid residues of phospholipids, the sugar residues

of cerebrosides and gangliosides, the sulfated sugar residues (with ionized SO_3^-) of sulfatides, and the OH group of free cholesterol. The nonpolar tails of membrane lipids include the hydrocarbon chains of fatty acid residues and sphingosine, and the sterol ring and side-chain of cholesterol. The polar head-group of a phospholipid may either double-up like the head of a walking stick (*Finean model*) or curve less acutely like the end of a golf club (*Vandenheuvel model*) around the contiguous ends of its nonpolar tails.

Phosphoglycerides mostly carry a long-chain saturated acyl group and a *cis*-polyenoic acyl group on respectively the α -C and β -C of glycerol residue. The staggered and more-or-less unbent hydrocarbon chains of saturated acyl groups are closepacked and hydrophobically bonded to each other. But the hydrocarbon chain of an unsaturated fatty acid suffers considerable bending at each of its *cis* double-bonds (Fig. 17.4). So, the hydrocarbon chains of polyunsaturated acyl groups

are considerably bent due to their *cis* double-bonds; this reduces the packing and hydrophobic bonding of their tails with the neighbouring acyl tails. So, the mobility of membrane lipids rises with the rise in unsaturation of their acyl residues.

The sterol ring of cholesterol is lodged below the curved polar head-group of a neighbouring phospholipid while its short hydrocarbon sidechain extends towards the bilayer midline through the lipid monolayer. According to the Vandenheuvel model, the bends at the *cis* double-bonds make the polyunsaturated fatty acid tail of a neighbouring phospholipid to wind around the distal part of the cholesterol sidechain. Both the sterol ring and its sidechain are hydrophobically bonded to the fatty acid tails of neighbouring phospholipids. The 3-OH group of nonesterified cholesterol remains polar-bonded with the charged polar head-group of the phospholipid molecule curved over the sterol ring. All these factors enable

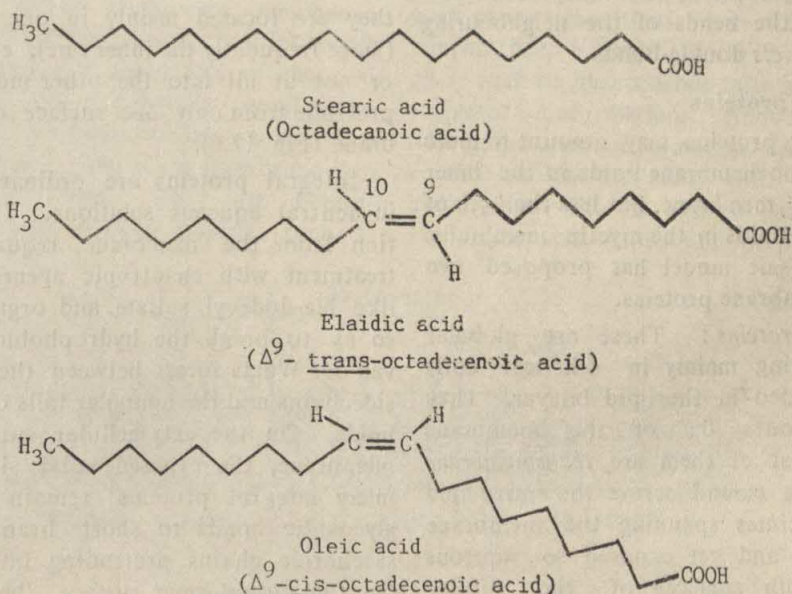


Fig. 17.4. Bending of the hydrocarbon chain of a *cis*-monounsaturated fatty acid in contrast to saturated and *trans*-unsaturated fatty acids.

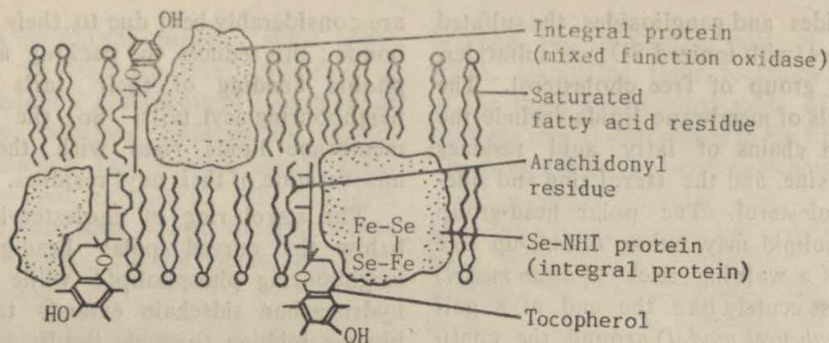


Fig. 17.5. Tocopherol molecules in membrane lipid bilayer.

cholesterol to make the packing of membrane lipids more compact and to decrease their mobility.

α -Tocopherol molecules occur in the lipid bilayer with their isoprenoid sidechain hydrophobically bonded to the nonpolar tails of neighbouring polyunsaturated fatty acids of membrane lipids. The chromane ring of tocopherol is oriented towards the water-adjointing surface of the bilayer (Fig. 17.5). The methyl groups of the tocopherol side-chain lie in the bends of the neighbouring acyl tail at its *cis* double-bonds.

Membrane proteins

Membrane proteins may amount to more than 3 times the membrane lipids in the inner mitochondrial membrane, but less than $\frac{1}{4}$ th of the membrane lipids in the myelin membrane. The fluid mosaic model has proposed two classes of membrane proteins.

Integral proteins: These are globular proteins existing mainly in α -helical coils deeply embedded in the lipid bilayer. They constitute about 70% of the membrane proteins. Most of them are *transmembrane proteins* which extend across the entire lipid bilayer, sometimes spanning the membrane several times, and get exposed to aqueous phases on both surfaces of the bilayer (Fig. 17.6). The ionizable amino and carboxyl terminals of transmembrane proteins may

project from opposite surfaces of the membrane (e.g., LDL receptors, cytochrome b_5 , transferrin receptor and glycophorin) or may both project from the cytoplasmic surface of the membrane (e.g., human glucose transporter). Transmembrane proteins are oriented asymmetrically in the membrane with different amino acid sidechains exposed on the two different surfaces. Some integral proteins are *nonspanning proteins*. These do not span the entire width of the bilayer; instead, they are located mainly in one monolayer (more frequently the inner one), extend partly or not at all into the other monolayer and protrude from only one surface of the membrane (Fig. 17.6).

Integral proteins are ordinarily insoluble in neutral aqueous solutions. Their extraction from the membrane requires drastic treatment with chaotropic agents, detergents like Na-dodecyl sulfate and organic solvents so as to break the hydrophobic bonds and van der Waals forces between their nonpolar sidechains and the nonpolar tails of membrane lipids. On the extracellular surface of the membrane, the exposed polar sidechains of many integral proteins remain bound by glycosidic bonds to short branched oligosaccharide chains protruding into the ECF. On the cytoplasmic surface, the polar sidechains of some integral proteins are bound to peripheral proteins by ionic bonds.

Peripheral proteins: These do not penetrate into the lipid bilayer. Instead, they remain loosely bound by ionic bonds to the exposed polar sidechains of specific integral proteins. They are thus located discontinuously on the cytoplasmic surface of the membrane, and are extracted from the latter by mild treatments with chelating agents, high ionic strengths or metal ions. They are soluble in neutral aqueous solutions and constitute about 30% of the membrane proteins. Through the gaps between peripheral proteins on the membrane surface, phospholipases can hydrolyze membrane phospholipids. Ankyrin of erythrocyte membrane and cytochrome c of inner mitochondrial membrane are peripheral proteins.

Synthesis and glycosylation of membrane proteins: See pages 497-503.

Membrane carbohydrates

Membrane carbohydrates consist mainly of short, branched hydrophilic oligosaccharide sidechains of integral glycoproteins and glycolipids. In the erythrocyte membrane, only about 15% of the oligosaccharides occur as glycoproteins and about 85% of them as glycosphingolipids, particularly gangliosides. The membrane oligosaccharides project from the extracellular surface of the membrane into the extracellular fluid. They are heteroglycans composed of sugar residues such as

sialic acids (NeuAc), L-fucose, N-acetylhexosamines and D-galactose. The COO^- groups of their NeuAc residues contribute negative surface charges on the cell; treatment with neuraminidase removes the NeuAc residues and lowers the surface charges and electrophoretic mobility of cells. Smaller amounts of membrane carbohydrates consist of D-glucose or D-galactose residues of membrane cerebrosides, and O-sulfated galactose residues of sulfatides.

Membrane fluidity

Membrane fluidity provides for the evagination, invagination, pinching off and fusion of membranes, reorientation, reorganization, incorporation and removal of segments of the membrane, flow of membrane molecules along the membrane, and their interactions.

Fluidity of the lipid bilayer: Membrane fluidity depends largely on the fluidity of the lipid bilayer.

(a) Transition temperature: This is the temperature at which a membrane lipid undergoes phase transitions. Below its transition temperature, a membrane lipid exists in a *gel phase* with its hydrocarbon tails in continuous staggered conformations. Above its transition temperature, a membrane lipid exists in a more mobile *sol phase* with many of its hydrocarbon tails in skewed conformations. The higher the proportion of phospholipids

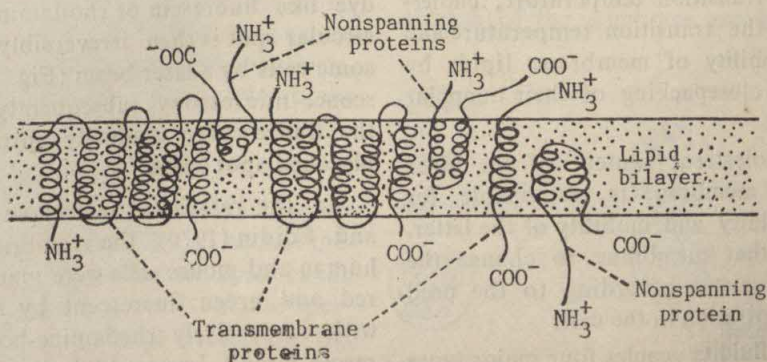


Fig. 17 6. Insertion and orientation of integral proteins in the membrane.

with low transition temperatures in the lipid bilayer, the greater is the membrane fluidity. Most membrane lipids ordinarily remain in the sol phase at physiological temperatures.

(b) *Unsaturated and lower fatty acids*: Because the hydrocarbon tail of an unsaturated fatty acid is considerably bent at each *cis* double-bond (Fig. 17.4), the presence of such fatty acids in membrane lipids reduces close-packing and hydrophobic interactions of fatty acid tails in the lipid bilayer. So, the membrane fluidity rises and the transition temperature of the membrane lipids falls with the rise in number and double-bonds of unsaturated fatty acid residues. Prolonged cold exposure increases the number of polyunsaturated acyl residues in membrane phospholipids; this lowers their transition temperature and maintains the membrane fluidity inspite of the low temperature. The short hydrocarbon tails of lower fatty acid residues also reduce the closepacking of fatty acid tails to increase the membrane fluidity.

(c) *Membrane cholesterol*: When the temperature exceeds the transition temperature, cholesterol elevates the transition temperature and reduces the mobility of membrane lipids by increasing the close-packing and hydrophobic interactions of the nonpolar tails of lipids. In this condition, the higher the cholesterol content, the lower is the membrane fluidity. But when the temperature is lower than the transition temperature, cholesterol reduces the transition temperature and raises the mobility of membrane lipids by decreasing the closepacking of their nonpolar tails.

The low cholesterol content of the inner mitochondrial membrane is responsible for the higher fluidity and mobility of the latter. This enables that membrane to change the density of its cristae according to the need for aerobic respiration in the cell.

Membrane fluidity enables four major types of movements of membrane lipids inside the

membrane. (i) *Lateral diffusion* of a lipid within the same monolayer at the average rate of $2 \mu\text{m sec}^{-1}$, taking about 10^{-17} sec for a shift to the next position. (ii) *Rotational diffusion*, i.e., rotation of a lipid molecule around its axis without any lateral shift. (iii) *Flexion* of the nonpolar tail in about 10^{-9} sec without change in location. (iv) *Flip-flop movement* from one monolayer to the other, at a rate many times slower than the lateral diffusion.

Mobility of membrane proteins: Membrane proteins can considerably diffuse laterally within the membrane. This rises with the fluidity of the lipid bilayer and the fall in the MW of the protein. Lateral diffusion of membrane proteins is restrained by (i) their existence as large oligomeric aggregates, (ii) their anchorage to oligosaccharides and peripheral proteins on the membrane surfaces, (iii) hydrophobic interactions and van der Waals forces between the nonpolar amino acid sidechains and nonpolar acyl tails of membrane lipids, (iv) sequestration or exclusion of some proteins to or from some domains of membrane lipids, and (v) the inability of membrane proteins to cross tight junctions between plasma membranes of adjacent cells.

In *fluorescence recovery after photobleaching* or FRAP technique, membrane proteins are first stained on cells by a fluorescent dye like fluorescein or rhodamine, and a small circular spot is then irreversibly bleached on some cells by a laser beam (Fig. 17.7). Fluorescence microscopy subsequently reveals that the fluorescence gradually spreads over the bleached spot again.

In the *cell fusion* technique used by Frye and Edidin (1970), the membrane proteins of human and mouse cells were made respectively red and green fluorescent by treating them with respectively rhodamine-bound and fluorescein-bound immunoglobulins. On treating these cells with polyethylene glycol or inactive

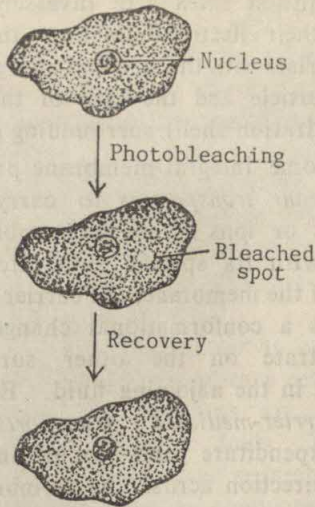


Fig. 17.7. Fluorescence recovery after photobleaching.

sendal virus and mixing them together, cells of the two species fused with each other into single hybrid cells or heterokaryones with a common plasma membrane. The red and green fluorescences first remained restricted to the respective halves of the heterokaryone membrane, but in about 40 minutes got uniformly distributed and intermixed over the entire fused membrane (Fig. 17.8).

Such findings indicate a lateral diffusion of the fluorescent proteins in the plane of the membrane.

While some membrane proteins move by lateral diffusion along the lipid bilayer at almost the same rate as the membrane lipids, others remain almost immobile. Membrane proteins may not make flip-flop movements.

Membrane asymmetry

Following factors cause a transverse or inside-outside asymmetry of the membrane.

(i) Asymmetric distribution of lipids in the two lipid monolayers. (ii) A higher percentage of unsaturated acyl groups in the inner monolayer than in the outer one. (iii) Asymmetric locations of peripheral proteins

and oligosaccharides on respectively the cytoplasmic and extracellular surfaces of the membrane. (iv) Asymmetric orientation of transmembrane proteins across the bilayer; e.g., the location of the ouabain-binding site of $\text{Na}^+\text{-K}^+\text{-ATPase}$ on the extracellular surface only. (v) Asymmetric location of non-spanning proteins; e.g., adenylate cyclase in the inner monolayer alone.

Transverse asymmetry is essential for unidirectional active transports across the membrane, enzyme activities on specific surfaces of the membrane, polarized states of membrane and location of molecular receptors for hormones and neurotransmitters on the extracellular surface.

Functions of membranes

(a) Membranes compartmentalize the cell.

The plasma membrane separates the entire protoplasmic mass from the extracellular medium. Membranes of organelles segregate specific collections of biomolecules in specific subcellular compartments like the nucleus and mitochondria. Membranes thus help to locate specific collections of molecules and specific metabolic reactions in specific cellular compartments.

(b) Many molecules including membrane proteins may flow along the membranes from one part of the cell to another.

(c) Due to the fluidity and mobility, stretches of the membrane may be evaginated,

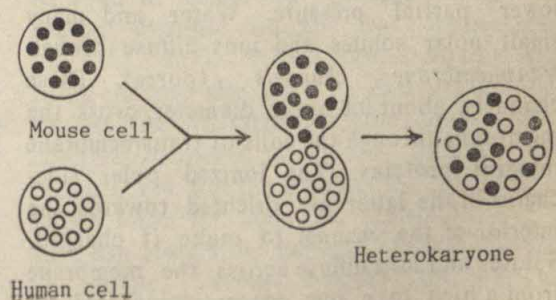


Fig. 17.8. Cell fusion technique.

invaginated, pinched off or fused with each other. This helps in cellular movements through the formation of filopodia and pseudopodia, renewal of the membrane, exocytosis and endocytosis.

Exocytosis is the extrusion of particulate or macromolecular materials which cannot pass through the intact membrane. A membrane-bound secretory vesicle, containing the secretory materials in its lumen, is pinched off from the membrane of Golgi cisternae; it marginates in the cell, extrudes its contents into the ECF through a fusion of its membrane with the plasma membrane and simultaneously adds its membrane to the latter.

Endocytosis is the cellular intake of extracellular materials. It is called *phagocytosis* or *pinocytosis* according to whether particulate or liquid materials are taken in (see pages 202, 213, 237, 244 and 448). A stretch of the plasma membrane is invaginated and pinched off to form a membrane-bound *endocytotic vesicle*, enclosing some water and some specific solutes or particles from the ECF. Simultaneously it removes a stretch of the plasma membrane. The vesicle may ultimately fuse with either a lysosome or a Golgi cisterna.

(d) Membranes are *selectively permeable*. They allow some materials to diffuse across them, but not the others. Lipid-soluble nonpolar materials like respiratory gases diffuse through the lipid core of the membrane; a gas diffuses from the side with its higher partial pressure to that with its lower partial pressure. Water and many small polar solutes and ions diffuse through *transmembrane channels* (pores). These channels, about 0.8 nm in diameter, cross the membrane through the coils of transmembrane integral proteins. The ionized polar side-chains of the latter are oriented towards the interior of the channel to make it charged. Solute and ions diffuse across the membrane from a high to a low concentration and towards the side that has an opposite charge.

Their diffusion rates are inversely proportional to their effective diameters—the effective diameter rises with the size and charge density of the particle and the size of the shell of water (hydration shell) surrounding it.

(e) Some integral membrane proteins act as *membrane transporters* to carry specific molecules or ions across the membrane. On binding with its specific substrate on one surface of the membrane, the carrier molecule undergoes a conformational change to bring the substrate on the other surface and releases it in the adjoining fluid. Frequently, such *carrier-mediated transports* involve energy expenditure and occur only in a specific direction across the membrane even against the concentration gradient of the substrate (*active membrane transport*); e.g., active outward transport of Na^+ by the sodium pump of the membrane (see pages 126-127). But sometimes such membrane transports require no energy expenditure and occur in either direction depending on the concentration gradient (*facilitated diffusion*); e.g., the membrane transport of fructose and mannose by intestinal cells (see pages 200-201).

Exocytosis, endocytosis, selective diffusion and carrier-mediated transports help in selective exchanges of materials across the membrane and thus maintain a specific collection of materials in the cytoplasm and organelles, in contrast to the random distribution of materials in the media outside the membrane.

(f) Many integral proteins act as *molecular receptors* in the membrane for specific hormones, neurotransmitters and drugs; e.g., adrenergic receptors, acetylcholine receptors and glucagon receptors. They bind with specific informational molecules reaching the membrane through the ECF and mediate in the cellular effects of those materials. The membrane thus participates in the *reception and transmission of information* from the extracellular medium.

(g) Many integral proteins function as *enzymes*; e.g., adenylate cyclase, guanylate cyclase and $\text{Na}^+\text{-K}^+\text{-ATPase}$ of the plasma membrane; respiratory chain enzymes of the inner mitochondrial membrane; prostaglandin synthase, fatty acid desaturases, fatty acid elongase, glycosyl transferases, squalene epoxidase and oxidosqualene cyclase of endoplasmic reticular membranes; mannosidase, fucosyl transferase and sialyl transferase of Golgi membranes.

(h) A *membrane capacitor* is constituted by the location of the membrane between two aqueous electrolyte solutions, viz., the extracellular and intracellular fluids. The membrane acts as a dielectric and separates the two fluids which behave as conductors. This membrane capacitor participates in maintaining the resting membrane potential. The $\text{Na}^+\text{-K}^+\text{-ATPase}$ actively transports three Na^+ ions outward and two K^+ ions inward across the membrane which is poorly permeable to Na^+ in the resting condition. This keeps the resting membrane *polarized* with its inner side slightly electronegative compared to its outer side and produces the negative membrane potential. Stimulation manifold increases the membrane permeability to Na^+ . The consequent inflow of Na^+ by diffusion down its inward electrochemical gradient across the membrane results in the depolarization of the membrane and initiates the action potential.

(i) Plasma membranes of adjacent cells may be modified at places to form lines, bands or disc-like areas of firm intercellular attachments called *cell junctions*.

(j) Polyunsaturated fatty acids of membrane phospholipids are used in the *synthesis of prostaglandins, thromboxanes and leukotrienes*.

(k) The oligosaccharide sidechains of some membrane proteins and glycolipids possess *antigenic properties*; e.g., blood group antigens and human leucocyte antigens.

17.2 SODIUM AND POTASSIUM GATES

Different ions diffuse through different transmembrane channels. Channels for cations are negatively charged internally and differ in charge density and cross section. Na^+ and K^+ diffuse through separate and highly selective sodium and potassium channels having maximum conductances of respectively 10^7 Na^+ ions and 10^6 K^+ ions per sec. The diffusion of a cation through the membrane is regulated by the transient opening or closure of a positively charged *gate* of its specific channel. The sodium and potassium channels are *voltage-gated channels* because their gates are narrowed or opened by changes in the polarization and potential of the membrane. A high *gating potential* around the channel narrows or closes such gates in the polarized membrane; the gates are opened by a fall in the gating potential resulting from a capacitative change in the depolarized membrane. A high gating potential probably blocks cation fluxes through the channels by electrostatic repulsion while a fall in that potential due to membrane depolarization enhances cation fluxes by probably weakening or abolishing that repulsion. The *sodium gate* is almost totally closed in the resting membrane, restricting Na^+ diffusion across the membrane to a very low level. But it is widely opened by membrane depolarization, enhancing by several thousand times the influx of Na^+ down its inward electrochemical gradient. The sodium gate is also opened by a fall in serum Ca^{2+} concentration. The sodium channel has an oval cross-section ($0.5 \times 0.3 \text{ nm}^2$). When open, a narrow negatively charged site near its extracellular end enables the hydrated Na^+ ion with a smaller effective diameter to diffuse through the channel, but not the hydrated K^+ ion with a larger effective diameter. Tetrodotoxin of fugu fish blocks the passage of Na^+ through the sodium channel because the cationic guanido group of tetrodotoxin binds com-

petitively with the negatively charged site of the channel. The *potassium gate* is already partly open in the resting membrane, maintaining a higher membrane permeability for K^+ than for Na^+ . Further opening of that gate by membrane depolarization enhances the outflow of K^+ down its outward concentration gradient by only fifty times and somewhat later than the rise in Na^+ influx. The potassium channel, circular in cross-section, is blocked by tetraethylammonium.

Another type of cation channel, almost equally permeable to Na^+ and K^+ , is one of the *ligand-gated channels* whose gates are opened or closed by the binding of specific ligand molecules with receptor proteins in the membrane. On binding with its specific receptor in the postsynaptic membrane, acetylcholine opens these cation channels to enhance both Na^+ inflow and K^+ outflow through them down respectively the inward Na^+ gradient and outward K^+ gradient; but the Na^+ inflow far exceeds the K^+ outflow because of a steeper Na^+ gradient.

17.3 COLLAGENS

Collagens are extracellular fibrous scleroproteins. Their oligosaccharide prosthetic groups have galactose, mannose, N-acetylhexosamines, fucose, xylose and sialic acid residues, but no hexuronic acid. The oligosaccharides are bound to specific hydroxylysine residues by O-glycosidic linkages. The rod-like collagen molecule, about $0.26 \mu m$ long and 1.5 nm thick, consists of a *triple helix* which is a right-handed supercoil of three α peptide chains. Each α -chain is in turn coiled into a left-handed helix with 3 amino acid residues in each turn of the coil (see page 64). The α -chains are held together by interchain hydrogen bonds, aldol crosslinks, secondary peptide bonds and amide bonds. Collagens are deficient in tryptophan, tyrosine, cysteine, cystine and methionine, but contain abundant numbers of glycine,

proline, hydroxyproline, alanine and hydroxylysine residues—about 21-23% of the amino acid residues consists of proline plus hydroxyprolines while every third amino acid residue is glycine. Each α -chain is made of more than 300 repeating tripeptide units of the type Gly-X-Y; X and Y are non-glycine amino acid residues of this unit and constitute the basic difference in the amino acid composition between different classes of α -chains. Many proline and some 3-hydroxyproline residues occur in X positions in the Gly-X-Y units while many 4-hydroxyproline and some hydroxylysine residues occupy Y positions. The tiny sidechains of glycine residues of the three α -chains lie buried in the interior of the narrowest part of the triple helix. The inflexibility of the rings of proline and hydroxyproline and the numerous covalent and noncovalent crosslinks between the α -chains stabilize the triple helix (see page 64).

Many triple helical molecules are bundled together in longitudinally staggered fashions to form a collagen fibril of 10-100 nm diameter. Inside each fibril, the ends of consecutive triple helices are separated by short gaps, but are overlapped by the surrounding triple helices. Combinations of different classes of α -chains form the triple helices of different types of collagen fibrils. Many collagen fibrils are crosslinked by covalent aldol bonds, secondary amide and peptide bonds and Schiff base formation so as to constitute a collagen fibre.

According to the microscopic structure, physicochemical properties and triple helical molecules, collagen fibres are divided into five types. *Type 1 collagen* is most widely distributed. It occurs in the areolar connective tissue of skin and fascia, white fibrous tissue of fibrous joints, and the intercellular matrix of fibrocartilages, tendons, ligaments, bones and dentin. In electron micrographs, its thick fibrils look regularly cross-banded

with a periodicity of about 64-67 nm due to thick *overlap zones* alternating with thin *hole zones* free from the overlap between the ends of the adjacent triple helical molecules. The latter are made of either two $\alpha 1(I)$ and one $\alpha 2(I)$ peptide chains or three $\alpha 1(I)$ chains, and are poor in hydroxylysine residues and oligosaccharides. *Type II collagen* occurs in the vitreous body and the intercellular matrix of hyaline cartilage. Its fibrils are finer, non-crossbanded, arranged in interlacing networks and rich in glycosylated hydroxylysine residues. Its molecules are made of three $\alpha 1(II)$ peptide chains. *Type III collagen* occurs as a fine network of 'reticulin' fibres in the reticular connective tissue forming the supporting framework of lymph glands, uterus, GI tract, liver, endocrine glands and blood vessels. Its fibres are stained with silver nitrate. The fibrils are fine, non-crossbanded, rich in hydroxyproline residues and made of triple helices containing three $\alpha 1(III)$ peptide chains, but have few glycosylated hydroxylysine residues. *Type IV collagen* occurs as a fine network of fibres in the basal lamina of the basement membranes of epithelia and endothelia and in the lens capsule. The triple helical molecules of its fibrils are made of $\alpha 1(IV)$ and $\alpha 2(IV)$ peptide chains which are very rich in glycosylated hydroxylysine and also relatively rich in 3-hydroxyproline, but poor in alanine residues. *Type V collagen* occurs in small amounts in the basal lamina of vascular endothelia and in bones and cartilages. It is made of $\alpha 1(V)$, $\alpha 2(V)$ and $\alpha 3(V)$ peptide chains, rich in glycosylated hydroxylysine, but poor in alanine residues.

17.4 CYTOSKELETON

A cytoplasmic framework of protein-made filaments and tubules constitute what is called the cytoskeleton. It provides the structural support, holds organelles in position, and participates in the intracellular translocation

of organelles and metabolites, ciliary, flagellar and microvillus movements, cell divisions, exocytosis and endocytosis. The constituents of the cytoskeleton are of three major types.

Microfilaments

These are slender, cylindrical, indefinitely long rods, averaging about 7 nm in diameter. They are composed mainly of double-helical molecules of a contractile protein called *F-actin*. Each F-actin molecule is made of two linear polymeric chains of globular and asymmetric *G-actin* molecules (MW 43 kdal). In nonmuscle cells, β and γ types of G-actin polymerize to form the F-actin filaments.

A fungal alkaloid *cytochalasin B* interacts with a specific end of actin microfilaments and consequently inhibits their polymerization into F-actin, thus causing their disappearance. The mushroom poison *phalloidin* blocks the functions of microfilaments by binding with F-actin and inhibiting its depolymerization.

In the resting nonmuscle cells, actin microfilaments form a network (*stress fibres*) in superficial cytoplasm beneath the plasma membrane. Microfilaments may form a lattice-like network in the cytoplasm and a thick band in the *belt desmosome* of the lateral plasma membrane; they may splay out into the cytoplasm from the belt desmosome to form a filamentous network called the

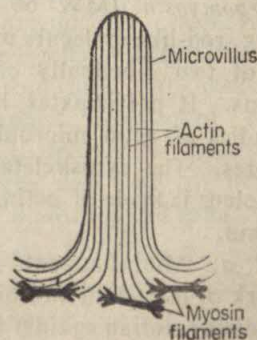


Fig. 17.9. Microfilaments in a microvillus.

adherens web. About 20-25 actin microfilaments run longitudinally inside each *microvillus* from the cytoplasm at its base and get attached to the plasma membrane at its tip (Fig. 17.9). In the migrating nonmuscle cells, the stress fibres are replaced by long cable-like bundles, oriented in the direction of movement. Microfilaments also extend into filopodia. In the dividing cell, microfilaments are concentrated and oriented initially along the mitotic spindle, and finally in the superficial cytoplasm near the cleavage furrow.

Actin microfilaments show structural polarity and possess a barbed end and a pointed end. *Myosin* filaments, thinner and shorter than those in muscle fibres, are associated with actin microfilaments at the cleavage furrow and also elsewhere. *Heavy meromyosin* binds specifically with the barbed end of actin microfilaments to form arrow-shaped actomyosin complexes.

Actin microfilaments remain regularly associated with still other proteins like α -actinin, filamin and tropomyosin. *Filamin* (MW 500 kdal) helps in the formation of a network of actin microfilaments. α -*Actinin* and *vinculin* occur in electron-dense plaques which anchor the ends of the actin microfilaments to the plasma membrane at the tips of microvilli and elsewhere. α -Actinin is made of a pair of identical peptides of MW 95 kdal. *Tropomyosin* (MW 66 kdal) has a 41 nm long rod-like molecule made by the interwinding of two α -helically coiled α and β peptide chains. It participates in forming cable-like bundles of actin microfilaments in the stress fibres. The cytoskeletal scaffold around the nucleus is made of actin, α -actinin and tropomyosin.

Functions: (a) Microfilaments form the inner framework of microvilli and bring about their movements by sliding against the myosin filaments at their base. (b) Microfilaments, attached to the plasma membrane and to the

membranes of organelles, serve to move them by sliding against the associated myosin filaments. This brings about the ruffling, evagination and invagination of the plasma membrane, formation of filopodia, pseudopodia and cleavage furrow, and intracellular translocation of organelles. (c) Similar actions of actin microfilaments of belt desmosomes close the gaps in epithelia resulting from the loss of cells.

Intermediate filaments

These are indefinitely long, thin rods, averaging 10 nm in diameter and occurring particularly in those parts of the cytoplasm where the cell is subjected to mechanical stress. They are not denatured by cytochalasin B or colchicine; only specific proteases or strong ionic detergents may depolymerize them. Each intermediate filament possesses nonhelical domains at its two ends, and four α -helical domains alternating with β -pleated domains. Five major types of intermediate filaments have been identified.

(a) *Tonofilaments*: These are long slender rods of about 8 nm diameter. They are made of fibrous proteins called *keratins I and II* (MW 42-65 kdal). About 20 different polypeptides occur in keratins. Each filament is a heteropolymer of two or more types of different polypeptides. Tonofilament bundles run in wide loops or straight courses through the cytoplasm of many epithelial cells. They connect several *spot desmosomes* by successively traversing their cytoplasmic plaques on the plasma membrane.

Tonofilaments provide a tensile structural framework in association with spot desmosomes and prevent the distortion of the cell in spite of severe mechanical stress.

Keratin-made filaments form extensive crosslinked networks in the cytoplasm of stratum corneum cells of epidermis and make them impermeable to water.

(b) *Neurofilaments*: These are long filaments, having a tetragonal cross-section and about 10 nm thickness, and enclose a less dense central core or lumen. Each neurofilament is a heteropolymer of three species of proteins of MW 68, 150 and 200 kdal respectively. Neurofilaments form a network of crosslinked bundles in the cytoplasm of the perikaryon, axon and dendrites of neurons to provide them a structural framework.

(c) *Glial filaments*: These 8-nm filaments are made of the polymers of a *glial fibrillar acidic protein* (GFAP) of MW averaging 50 kdal. Crosslinked bundles of these filaments form a structural network in the cytoplasm and run into the processes of neuroglia cells, particularly astrocytes.

(d) *Vimentin filaments*: These 10-nm filaments occur in the cytoplasm of a wide variety of mesenchymal and nonmesenchymal cells. They are composed of a polymer of the protein *vimentin* (MW 52 kdal), but are sometimes heterogeneous, carrying desmin and GFAP in addition. Vimentin filaments form a network on the cytoplasmic surface of the plasma membrane, often get anchored to both the plasma membrane and the nuclear envelope, and may hold the latter in position.

(e) *Desmin filaments*: These 10-nm non-contractile filaments are made of the protein *desmin* (50 kdal). They occur in the Z bands of muscle fibres and hold together the contractile protein filaments.

Microtubules

These are unbranched, hollow, cylindrical tubules, several μm long and about 25 nm in diameter. They course in more or less straight paths through the cytoplasm. Each microtubule has an electron-lucent central core or lumen, measuring about 17 nm in diameter and surrounded by a 4-5 nm thick dense wall made of 13 longitudinal strands or *protofilaments*. Each protofilament is a

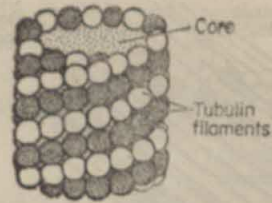


Fig. 17.10. Microtubule.

linear chain of many heterodimers of two globular proteins, α -tubulin (MW 53 kdal) and β -tubulin (55 kdal). The tubulins are roughly spherical and about 4 nm in diameter. So, each heterodimer ($\alpha\beta$) is about 8 nm long. Many such dimers are joined serially lengthwise to form a protofilament. The latter carries alternating α and β tubulins along its length and ends with an α -tubulin at one end and a β -tubulin at the other. Each type of tubulin molecules appears to occur in left-handed helical rows around the microtubule (Fig. 17.10). Small amounts of nontubulin proteins such as *tau* (MW 55-62 kdal) and *high-MW proteins* (>280 kdal) remain associated as projections on the cytoplasmic surface of microtubules and promote tubulin formation. The alkaloid *colchicine* of the autumn crocus, but not cytochalasin B, prevents the polymerization of tubulins into protofilaments and consequently dissolves the microtubules.

Microtubules run either singly or in bundles through the cytoplasm of almost all eukaryotic cells. They often spread out radially from near the centrioles. Microtubule bundles, crosslinked with neurofilaments and frequently called *neurotubules*, traverse the cell body of neurons and the lengths of dendrites and axons.

Microtubules occur in two ways in the mitotic spindle—*kinetochore microtubules* extend from the kinetochores of chromosomes to the spindle poles, linking the chromatids directly to the spindle poles, while *interpolar*

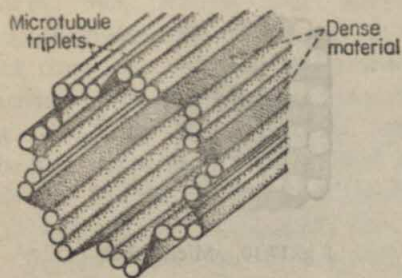


Fig. 17.11. Centriole.

microtubules run between the two poles without any connection to chromosomes and are made of overlapping discontinuous segments

Each *centriole* is a cylinder (about $1\ \mu\text{m} \times 200\ \text{nm}$) bearing 9 circumferential triplets of microtubules parallel to its long axis (Fig. 17.11). The three microtubules of each triplet, called A, B and C subfibres, are partly fused with each other and set along a plane, making a variable angle with the circumference of the cylinder. The innermost A subfibre is made of 13 longitudinal tubulin protofilaments; the middle subfibre B bears 10 protofilaments of its own and shares three

more of A; the outermost subfibre C shares three protofilaments of B besides itself carrying 10 protofilaments. Each subfibre A is connected to one radial spoke running towards the central axis of the centriole. This arrangement forms a *cartwheel structure* at one end of the latter.

The *basal body*, situated in the cytoplasm at the base of each cilium, is a modified centriole and is made of microtubules. In each *cilium* or *flagellum*, 9 pairs of partly fused microtubules, elliptical in cross-section, run longitudinally in its peripheral cytoplasm while two single and wider microtubules, circular in cross-section, run near its central axis (Fig. 17.12). The central microtubules are encased by a *central sheath* made of spiral coils of a fibre. The subfibres A and B of each peripheral doublet are made of 13 and 10 tubulin protofilaments each, three protofilaments of A being shared in addition by B. Each subfibre A has a radial arm ending in a hammer-like head near the central microtubules, and also sends towards the subfibre B of the next set a pair of arms made of a protein *dynein* (MW $\approx 500\ \text{kDa}$)

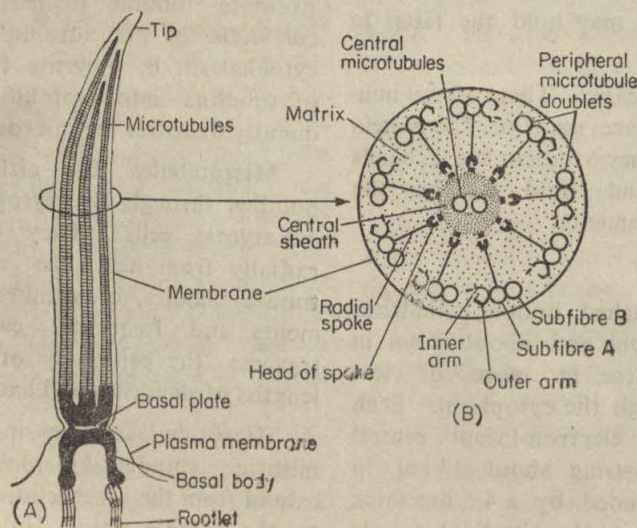


Fig. 17.12 Cilium.

having ATPase activity. A flexible band of the protein *nexin* interconnects the peripheral doublets.

Centrioles, basal bodies and kinetochores may serve as *nucleation centres* for the polymerization of tubulins in forming microtubules.

Functions : (a) Movements of cilia and flagella result from a sliding of their microtubule doublets against each other. (b) Progressive depolymerization of tubulin protofilaments shortens the microtubules connected with the chromatids and thereby causes the migration of the chromatids towards the spindle pole during karyokinesis. (c) Microtubules are constituents of cytoskeleton and help to maintain the shapes of cells, cilia, flagella, dendrites and axons. (d) Microtubules may function as rails along which organelles may pass from one cellular site to another. (e) They may also serve as tubules to conduct chemical substances along their central lumen.

17.5 CELL JUNCTIONS

Cell junctions are formed between adjacent cells by the interaction, attachment and modification of some areas of their plasma membranes.

Tight junction or zonula occludens

A tight junction consists of several interlinked lines of attachment (*sealing strands*)

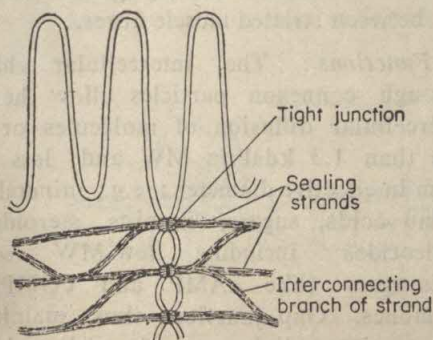


Fig. 17.13. Tight junction.

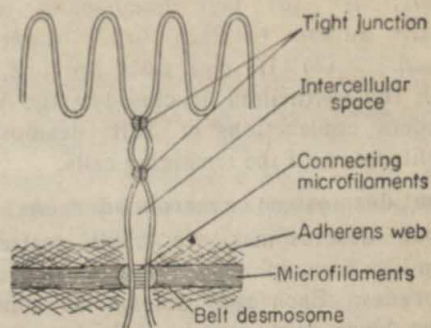


Fig. 17.14. Belt desmosome.

between the lateral plasma membranes of adjacent cells close below their free surface (Fig. 17.13). The lipid bilayers of both membranes are totally fused along the sealing strand and pairs of *inverted micelles* are formed in the fused middle core of the bilayer (pages 36-37). Tight junctions occur between the brush-bordered cells in the intestinal mucosa and renal proximal tubules, between the endothelial cells in brain, and between the presynaptic and postsynaptic membranes around the synapse.

Functions : (a) Tight junctions join the adjacent plasma membranes so tightly as to prevent diffusion of molecules and ions along the intercellular space across such junctions (*impermeable junctions*). (b) Tight junctions also block the random lateral diffusion of integral proteins along the membrane.

Belt desmosome or zonula adherens

It is a band-like zone of adherence between the lateral plasma membranes of columnar epithelial cells and runs parallel to their free surface (Fig. 17.14). It consists of two bundles of actin-made microfilaments, located on the cytoplasmic surfaces of the membranes and interlinked by fine microfilaments across the intervening intercellular space. The microfilaments are connected with those of the *adherens web*.

Functions: (a) Belt desmosomes make the cells adhere to each other (*adherent junctions*). (b) If some cells are lost, the gap in the epithelium is closed by the ATP-dependent contractions of belt desmosome microfilaments of the remaining cells.

Spot desmosome or macula adherens

Spot desmosomes are small, scattered, button-like areas of adherence between plasma membranes. Each spot desmosome consists of two dense, oval, protein-made *cytoplasmic plaques* (500 nm × 300 nm), located on the cytoplasmic surfaces of the two membranes and interconnected across the intercellular space by thin filamentous *transmembrane linkers* (Fig. 17.15). The linkers are linked in a staggered manner to a dense, discontinuous *central stratum* which bisects the mucoprotein filamentous material in the intercellular space. Very long, cylindrical, rod-like, keratin-made *tonofilaments* loop from the cytoplasm through the plaques.

Functions: Spot desmosomes form multiple points of adherence between cells of uterine cervix, cardiac muscle, skin, etc., and prevent their displacement and distortion in spite of severe mechanical stress.

Gap junction or nexus

A gap junction is a disc-shaped area on each of two adjacent plasma membranes

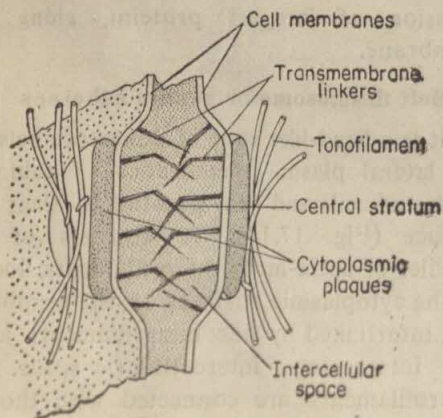


Fig. 17.15. Spot desmosome.

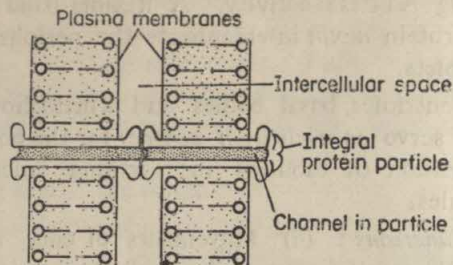


Fig. 17.16. Gap junction.

separated by a narrow intercellular space. At the gap junction, each membrane carries many hexagonal cylindrical particles called *connexons* (MW 18 kdal), each a hexagonal tube-like aggregate of 6 dumbbell-shaped subunits surrounding a central channel of 1.5-2 nm diameter (Fig. 17.16). The subunits are inclined against the lipid bilayer of the membrane; a rotation of the subunits causes them to slide past each other so as to obliterate the orifice of the central channel of the connexon (Fig. 17.17). Each connexon particle spans the entire width of the membrane and protrudes into the intercellular space to abut against a connexon of the other membrane. The central channels of the two particles consequently form a continuous intercellular tubule across both membranes.

Gap junctions occur abundantly between cardiac muscle cells, visceral smooth muscle cells, neurons and salivary gland cells, but not between striated muscle fibres.

Functions: The intercellular channels through connexon particles allow the direct intercellular diffusion of molecules or ions, less than 1.3 kdal in MW and less than 2 nm in effective diameter; e.g., mineral ions, amino acids, sugars, vitamins, steroids and nucleotides including low-MW second messengers (like cAMP and cGMP) for hormones. Gap junctions thus maintain a common intercellular pool of small molecules and ions in the coupled cells, but retain

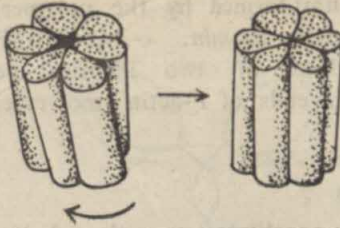


Fig. 17.17. Rotation of a connexon particle to close its central channel. [After P. N. T. Unwin and G. Zampighi, 'Structure of the junction between communicating cells', *Nature*, 283 : 545, 1980.]

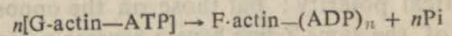
their individuality by preventing intercellular exchange of macromolecular structural proteins, polysaccharides, enzymes and nucleic acids. They also provide for the direct cell-to-cell transmission of impulses through ion diffusions. Their permeabilities are controlled by intracellular Ca^{2+} concentrations. Rise in intracellular Ca^{2+} concentration causes a clockwise rotation of the subunits of connexon at their base to reduce their inclination to the transmembrane axis, decreasing thereby the effective diameter of the gap junction channel. Thus, the channel is totally impermeable at Ca^{2+} concentrations exceeding 5×10^{-5} M, but fully permeable at Ca^{2+} concentrations below 10^{-7} M.

17.6 MUSCLE PROTEINS

Major muscle proteins include actin, myosin, troponin, tropomyosin, actinin, desmin, calsequestrin and myoglobin. [For *calsequestrin* and *myoglobin*, see pages 118 and 252-253.]

Actin

Actin constitutes about $\frac{1}{3}$ th of total muscle proteins. At low ionic concentrations and in absence of Mg^{2+} , actin exists as water-soluble, ATP-bound, globular monomers called *G-actin* (MW 43 kdal) made of 376 amino acids. In presence of Mg^{2+} and at isotonic or higher ionic concentrations, G-actin molecules polymerize with the breakdown of ATP to form insoluble, ADP-bound, elongated and beaded fibrous filaments of *F-actin* (Fig. 17.18).



F-actin filaments are double-stranded with the two strands coiled around each other to form α -helices. The F-actin double-strand is about 6-7 nm thick, carries about 14 G-actin molecules per turn and has a pitch of 35.5 nm. An F-actin double-strand, along with troponin and tropomyosin associated with it, forms a thin myofilament in the I band of myofibrils. F-actin double-strands also encroach for some distance into the A band, adjoining the I band, to interdigitate with the ends of thick myosin filaments of the A band. The other end of the F-actin double-strand remains attached to α -actinin molecules of the Z line between I bands of successive sarcomeres.

On addition of myosin, both F-actin strands of a thin myofilament acquire an arrowhead pattern along their entire lengths, all the arrows pointing away from the Z line to which the myofilament is attached. All thin filaments on one side of a Z line have an

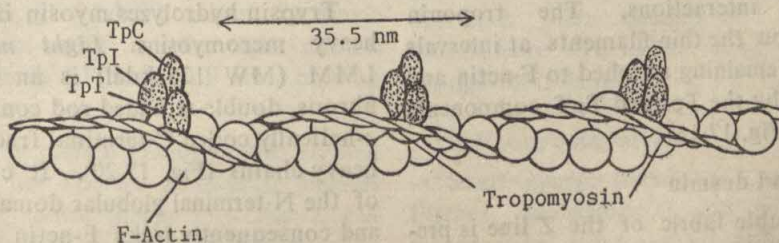


Fig. 17.18. A thin myofilament.

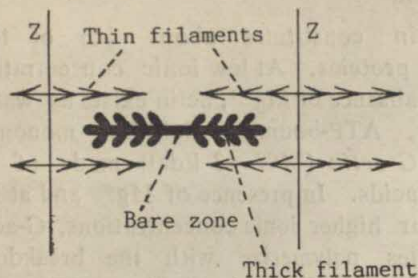


Fig. 17.19. Polarity of actin filaments.

identical polarity; but those on the opposite side of the same Z line have a reverse polarity (Fig. 17.19).

Tropomyosin

It has a rod-like fibrous molecule, about 41 nm long, about 66 kdal in MW and consisting of one each of α -helical α and β peptide chains. The double-stranded tropomyosin molecule lies almost parallel to the thin filament in the long helical groove between its F-actin strands and remains bound to the TpT subunit of troponin (Fig. 17.18).

Troponin

The troponin system (MW $\approx$ 80 kdal) consists of three interconnected globular proteins. (i) *TpT* (37 kdal) is a tropomyosin-binding protein of 259 amino acid residues. (ii) *TpC* (18 kdal) bears 4 Ca^{2+} -binding sites, consists of 159 amino acid residues and closely resembles *calmodulin* structurally and functionally. (iii) *TpI* (24 kdal) possesses binding sites for F-actin and *TpC*, consists of 179 amino acid residues and inhibits actin-myosin interactions. The troponin systems lie on the thin filaments at intervals of 38.5 nm, remaining attached to F-actin and tropomyosin by the *TpI* and *TpT* components respectively (Fig. 17.18).

Actinin and desmin

The insoluble fabric of the Z line is predominantly made of *intermediate filaments*

(diam. 10 nm) formed by the polymerization of the protein *desmin*. α -*Actinin* (MW 190 kdal), a dimer of two identical peptides, anchors the ends of F-actin molecules to the Z line.

Myosin

Myosin constitutes more than half of the total muscle proteins. It has a hexameric molecule (MW 460 kdal) made of two heavy and four light chains (Fig. 17.20). The heavy chains (200 kdal) form a double-stranded fibrous rod, 2 nm in diameter and 134 nm long, by coiling α -helically about each other except at their N-terminal ends where each forms a globular domain (11 nm $\times$ 4 nm) bound to two light chains (15-27 kdal). The terminal globular domains possess ATPase activity (*myosin-ATPase*) and binding sites for F-actin. Each thick filament of the A band consists of hundreds of parallel myosin molecules arranged in two oppositely directed bundles. Cross-bridges project in a helical order from the thick filament except from its 150-nm long middle segment called the *bare zone*. The myosin molecules on the two sides of the bare zone are oriented in opposite directions. The C-terminal ends of myosin molecules of both sets meet at the H zone in the middle of the A band while their N-terminal globular domains are directed towards the opposite margins of the A band. The globular domains of consecutive molecules of each set project at staggered angles from the thick filament and at 14.3 nm intervals along its long axis.

Trypsin hydrolyzes myosin into light and heavy meromyosins. *Light meromyosin* or LMM (MW 125 kdal) is an 85 nm long, fibrous, double-stranded rod consisting of the α -helically coiled C-terminal fractions of two heavy chains (Fig. 17.20). It carries neither of the N-terminal globular domains of myosin and consequently lacks F-actin binding sites and ATPase activity. *Heavy meromyosin* or

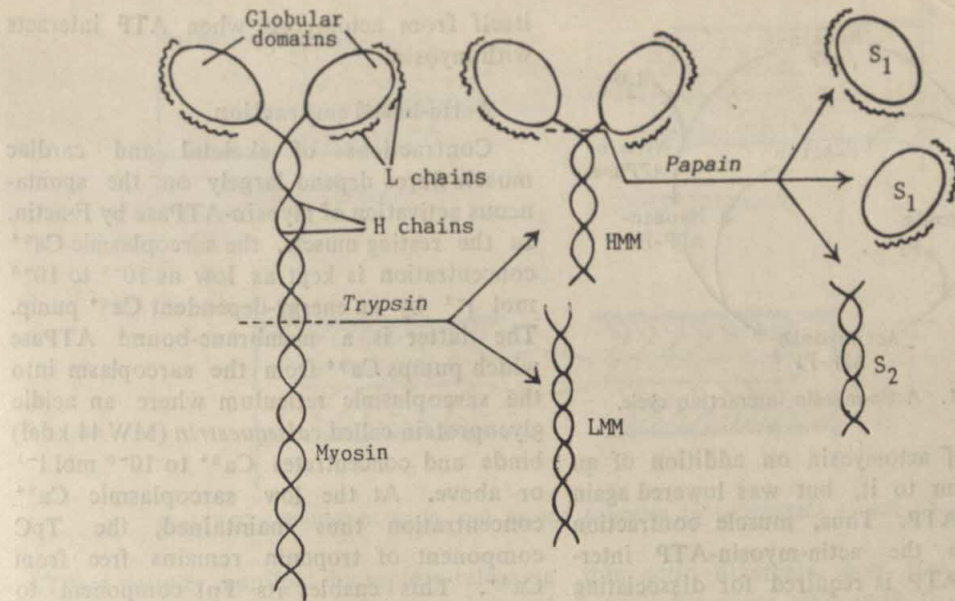


Fig. 17.20. A myosin molecule and its enzymatic cleavage into fragments.

HMM (340 kdal) consists of the N-terminal ends of two heavy chains, α -helically coiled about each other for a 40 nm stretch and bearing the globular domains with their associated light chains. Because of the globular domains, HMM retains both F-actin binding ability and ATPase activity. Cross-bridges project from the HMM portion only.

Papain hydrolyzes HMM further into a fibrous S_2 fragment and two globular S_1 fragments. S_2 consists of the α -helical, double-stranded C-terminal fraction of HMM and lacks ATPase activity and F-actin binding sites. Each S_1 (115 kdal) consists of a single globular domain bound to two light chains, and possesses both ATPase and F-actin binding activities.

Trypsin and papain hydrolyze the myosin molecule at its specific nonhelical flexible *hinge regions*. Such hinges join the LMM domain to the S_2 domain and the latter to the S_1 domains. These hinges make the molecule flexible at the S_1 - S_2 and S_2 -LMM junctions

and thereby enable the S_1 domains to tilt and rotate for precise interactions with actin filaments during muscle contraction.

17.7 MUSCLE CONTRACTION

According to the *sliding filament model* of muscle contraction, the thin actin filaments slide towards the centre of the A band and between the thick myosin filaments; this increases the overlap between the thin and thick filaments and shortens the muscle fibre. During relaxation, the thin filaments slide out from between the thick filaments; this decreases their overlap and elongates the muscle fibre. In neither case, the filaments change their lengths.

Actin-myosin interaction

Szent-Gyorgi found that unlike myosin threads, actomyosin threads contracted when placed in a solution containing ATP, Mg^{2+} and K^+ . He also showed that the viscosity of a myosin solution was enhanced by the

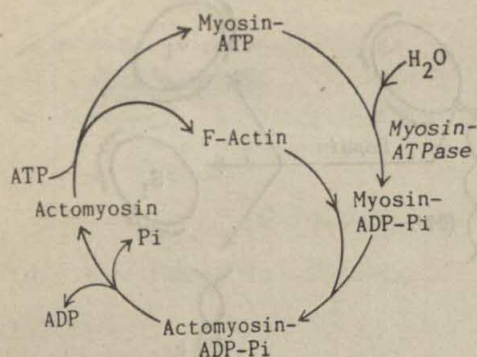


Fig. 17.21. Actin-myosin interaction cycle.

formation of actomyosin on addition of an actin solution to it, but was lowered again on adding ATP. Thus, muscle contraction results from the actin-myosin-ATP interaction and ATP is required for dissociating actomyosin during relaxation.

Myosin binds with ATP which is hydrolyzed to ADP and Pi by the myosin-ATPase, located in its S_1 domains and modulated by the associated light chains. But ADP and Pi cannot be released from myosin until F-actin binds with myosin. Thus, in the resting muscle fibre, myosin exists as the myosin-ADP-Pi complex (Fig. 17.21). During muscle contraction, actin binds with the S_1 domains of myosin in the myosin-ADP-Pi complex to form an actomyosin-ADP-Pi complex; this releases ADP and Pi from myosin at a high rate, leaving actomyosin behind. A fresh molecule of ATP next binds with the myosin part of actomyosin; this lowers the affinity of myosin for actin and consequently releases the latter from actomyosin. In the myosin-ATP complex left behind, myosin-ATPase hydrolyzes ATP to ADP and Pi which continue to remain bound to myosin as myosin-ADP-Pi, awaiting the next interaction with actin. In this way, actin-myosin interaction is repeated cyclically—actin alternately binds to myosin-ADP-Pi to release ADP and Pi and again gets released

itself from actomyosin when ATP interacts with myosin.

Actin-based contraction

Contractions of skeletal and cardiac muscle fibres depend largely on the spontaneous activation of myosin-ATPase by F-actin. In the resting muscle, the sarcoplasmic Ca^{2+} concentration is kept as low as 10^{-7} to 10^{-8} mol l^{-1} by an energy-dependent Ca^{2+} pump. The latter is a membrane-bound ATPase which pumps Ca^{2+} from the sarcoplasm into the sarcoplasmic reticulum where an acidic glycoprotein called *calsequestrin* (MW 44 kdal) binds and concentrates Ca^{2+} to 10^{-3} mol l^{-1} or above. At the low sarcoplasmic Ca^{2+} concentration thus maintained, the TpC component of troponin remains free from Ca^{2+} . This enables its Tpl component to inhibit the actin-myosin interaction either by rolling the TpT-bound tropomyosin into a position blocking the S_1 -binding sites of F-actin sterically, or by promoting a tropomyosin-mediated conformational change in F-actin. This keeps the myosin head detached from F-actin and the muscle fibre consequently remains relaxed with myosin existing as myosin-ADP-Pi complex only.

Stimulation of the muscle fibre depolarizes the T-tubule membrane. This triggers a large-scale Ca^{2+} influx from the sarcoplasmic reticulum to the sarcoplasm, raising the sarcoplasmic Ca^{2+} concentration to 10^{-5} mol l^{-1} . Ca^{2+} now binds with the TpC component of troponin to form $TpC.4Ca^{2+}$ which either alters the conformation of F-actin through tropomyosin or rolls tropomyosin into the centre of the long helical groove of F-actin, exposing the S_1 -binding sites of the latter. This nullifies the inhibitory action of Tpl on actin-myosin interaction and enables F-actin of thin filaments to bind with the S_1 domains of myosin heads of thick filaments to form actomyosin. This actin-myosin interaction accelerates myosin-

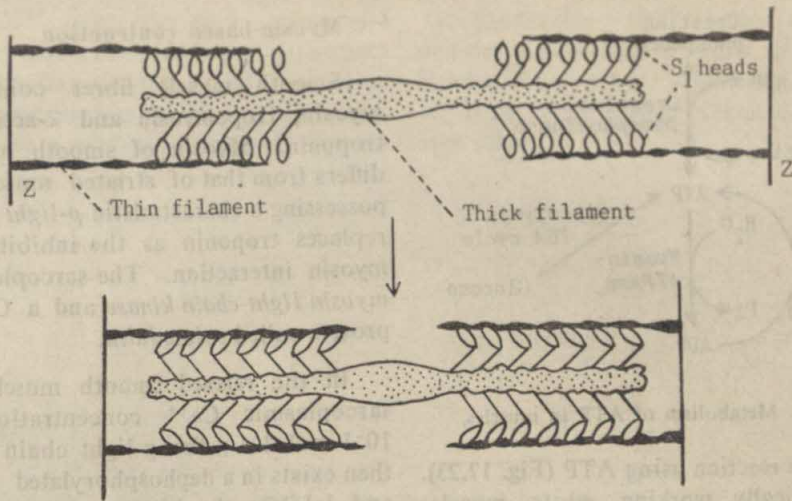


Fig. 17.22. Sliding of actin and myosin filaments in contracting muscle fibre.

ATPase activity manifold by rapidly releasing ADP and P_i held so long by myosin, and rotates the actin-linked myosin head to make a 90° angle with the long axis of the myofibril. The myosin head immediately rotates back to a 45° angle for attaining the minimum-energy conformation of the actin-myosin bond. Myosin consequently exerts a pull on actin. This causes a sliding of the thin actin filaments by about 7.5 nm towards the centre of the A band and between the thick myosin filaments, shortening the muscle fibre (Fig. 17.22).

A fresh molecule of ATP next binds to the S_1 head of myosin. This lowers the affinity of myosin for actin, detaching the S_1 head from actin and releasing the latter from actomyosin (Fig. 17.21). Simultaneously, Ca^{2+} is sequestered again into the sarcoplasmic reticulum by the Ca^{2+} pump and stored there as Ca^{2+} -calsequestrin. This lowers the sarcoplasmic Ca^{2+} concentration back to 10^{-7} M and helps to remove Ca^{2+} from TpC . $4Ca^{2+}$; TpC consequently can no longer prevent the inhibitory action of tropomyosin on actin-myosin interaction so that no fresh F-actin molecule can bind with the myosin head. The

thin actin filaments, now free from thick myosin filaments, slide away from the latter, elongating the fibre during its relaxation. Concomitantly, myosin-ATPase hydrolyzes the ATP, bound to myosin, to produce ADP and P_i which are left in combination with myosin until fresh actin molecules bind to the latter at the onset of the next contraction.

The reversible rotations of the S_1 heads of myosin and their interactions with F-actin are made possible by the flexible *hinge regions* between the S_1 heads and the S_2 domain of the HMM unit of myosin and between S_2 and the LMM unit.

Ca^{2+} acts as an allosteric activator of the actin-myosin interaction and muscle contraction, by binding with TpC and triggering conformational changes of the F-actin-tropomyosin-troponin complex.

Restoration of ATP: The ATP, hydrolyzed by myosin-ATPase during muscular activity, is promptly restored in the muscle in several ways. (a) *Creatine phosphokinase* transfers the high-energy phosphate group of creatine phosphate to ADP, producing ATP and creatine (*Lohmann reaction*). During recovery, creatine phosphate is regenerated

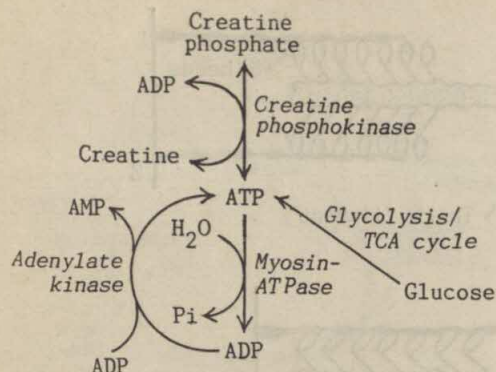


Fig. 17.23. Metabolism of ATP in muscles.

by the reverse reaction using ATP (Fig. 17.23). (b) Anaerobically working white muscles restore ATP largely by *substrate-level phosphorylations* during glycolysis, producing lactate. Glycolysis is enhanced by the allosteric activation of phosphofructokinase by ADP formed by myosin-ATPase action. (c) Aerobically working red muscles regenerate ATP largely by *oxidative phosphorylations* following the TCA cycle. (d) During prolonged activity, ATP is also restored by β -oxidation of fatty acids. (e) *Adenylate kinase* (myokinase) of muscles catalyzes the interactions of ADP molecules to produce ATP and AMP (Fig. 17.23).

Myosin-based contraction

Smooth muscle fibres contain F-actin, myosin, tropomyosin and α -actinin, but no troponin. Myosin of smooth muscle fibres differs from that of striated muscle fibres in possessing a characteristic *p-light chain* which replaces troponin as the inhibitor for actin-myosin interaction. The sarcoplasm contains *myosin light-chain kinase* and a Ca^{2+} -binding protein called *calmodulin*.

In the relaxed smooth muscle fibre, the sarcoplasmic Ca^{2+} concentration is below $10^{-7} \text{ mol l}^{-1}$; the p-light chain of myosin then exists in a dephosphorylated active form and inhibits the binding of F-actin to the myosin heads, preventing the contraction of the fibre. As the sarcoplasmic Ca^{2+} concentration rises to $10^{-5} \text{ mol l}^{-1}$ in the stimulated fibre, calmodulin binds with four Ca^{2+} to form $\text{calmodulin.4Ca}^{2+}$ which then binds with and activates the light-chain kinase (Fig. 17.24). The latter uses ATP to phosphorylate the p-light chain, inactivating it by *reversible covalent modification*. The phosphorylated p-light chain no longer inhibits the actin-myosin interaction. F-actin now binds with myosin heads, leading to a sliding of actin and myosin filaments towards each other.

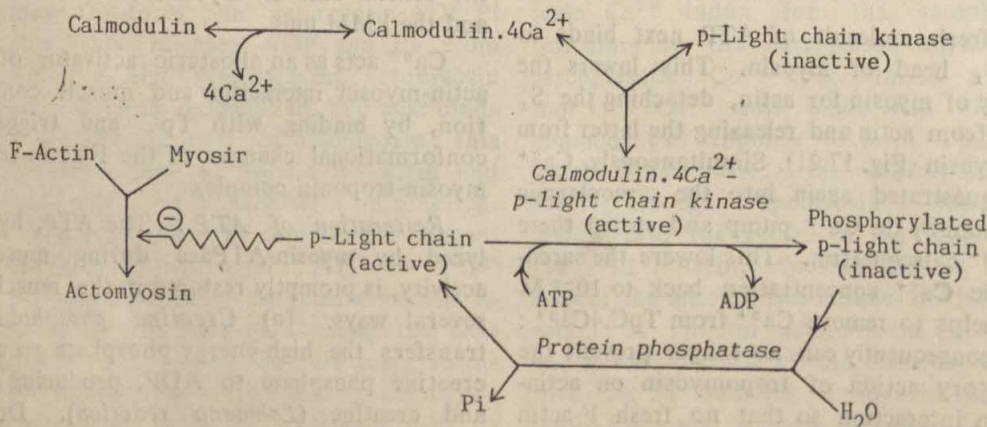


Fig. 7.24. Regulation of smooth muscle contraction.

As the sarcoplasmic Ca^{2+} falls during relaxation, Ca^{2+} dissociates from calmodulin which in turn dissociates from the light-chain kinase, inactivating the latter and preventing further phosphorylation of the p-light chain. Simultaneously, a *protein phosphatase* dephosphorylates the phosphorylated p-light chains.

The dephospho-p-light chains inhibit actin-myosin interaction to cause dissociation of F-actin from myosin. This results in a sliding of actin and myosin filaments away from each other.

12. HORMONES

Hormones are released by specialized endocrine secretory cells into the extracellular space and are then carried generally by blood to distant target cells (telecrine secretion). Sometimes hormones diffuse locally through the interstitial fluid from the secretory cells to the adjacent target cells (paracrine secretion). Testosterone, for example, acts as a paracrine hormone in stimulating spermatogenesis in seminiferous tubules. A third mechanism of action is the secretion of hormones which inhibit or stimulate other cells. Some neurons secrete hormones (neurohormones) into the blood for transport to their target cells (neuroendocrine secretion).

Group 1 hormones include the steroid hormones of the adrenal cortex and gonads, and the related amino acid hormones of the thyroid. These lipid-soluble hormones are mostly transported in free form in the plasma, but cannot readily enter the target cells through their membranes. They bind with specific receptor molecules in the plasma membrane. This triggers the formation of the cyclic AMP (cAMP) second messenger system. The cyclic AMP, in turn, activates the 1,3-bisphosphoglycerate dehydrogenase (1,3-BPGDH) which produces the cellular effect (2nd messenger). They affect the target cells by changing the membrane permeability, the rate of specific enzyme activities or the synthesis or degradation of certain proteins.

Group 2 hormones include the steroid hormones of the adrenal cortex and gonads, and the related amino acid hormones of the thyroid. These lipid-soluble hormones are mostly transported in combination with plasma proteins. With steroid hormones readily diffusing into the target cells through the membrane, they affect the target cells by changing the membrane permeability, the rate of specific enzyme activities or the synthesis or degradation of certain proteins.

Group 3 hormones include the steroid hormones of the adrenal cortex and gonads, and the related amino acid hormones of the thyroid. These lipid-soluble hormones are mostly transported in combination with plasma proteins. With steroid hormones readily diffusing into the target cells through the membrane, they affect the target cells by changing the membrane permeability, the rate of specific enzyme activities or the synthesis or degradation of certain proteins.

12.1 HORMONE ACTION

Group 1 hormones include the steroid hormones of the adrenal cortex and gonads, and the related amino acid hormones of the thyroid. These lipid-soluble hormones are mostly transported in combination with plasma proteins. With steroid hormones readily diffusing into the target cells through the membrane, they affect the target cells by changing the membrane permeability, the rate of specific enzyme activities or the synthesis or degradation of certain proteins.

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Hormones are *informational molecules*. They reach the information about changes inside or outside the body to specific target cells, enabling them to respond by changes in their activities. Hormones do not function as enzymes or coenzymes in catalyzing the formation or cleavage of covalent bonds in substrates. They affect the target cells by changing the *membrane permeability*, the rates of specific *enzyme activities* or the *synthesis or degradation of enzymes*.

18.1 HORMONE GROUPS

Group I hormones include the *steroid hormones* of the adrenal cortex and gonads, and the *iodinated amino acid hormones* of the thyroid. These lipid-soluble hormones are mostly transported in combination with polar plasma proteins. While steroid hormones readily diffuse into the target cells through

their membranes, thyroid hormones are carried in by active transport. They then bind with specific receptor molecules in the cytoplasm and/or the nucleus. The receptor-hormone complex mainly changes the rate of transcription of specific genes to produce the cellular effects.

Group II hormones include the *peptide and protein hormones* of the hypothalamus, pituitary, pancreas, gastrointestinal tract, parathyroids and placenta, and the *biogenic amines* such as the catecholamines of adrenal medulla, dopamine of hypothalamus and melatonin of the pineal. These water-soluble hormones are mostly transported in free forms in the plasma, but cannot readily enter the target cells through their membranes. They bind with specific receptor molecules in the plasma membrane. This triggers the formation in the cytosol of specific *second messengers* like cyclic AMP (cAMP), inositol 1,4,5-triphosphate and diacylglycerol, which in turn produce the cellular effects (*Sutherland's double-messenger theory*).

18.2 MECHANISM OF HORMONE ACTION

Each hormone initiates in its target cells specific molecular events which ultimately change the cellular activities.

Interaction with nuclear chromatin

Steroids and thyroid hormones act mostly by changing the transcription rate of specific genes in the nuclear DNA.

Entering the target cell through the membrane lipid bilayer, a steroid hormone

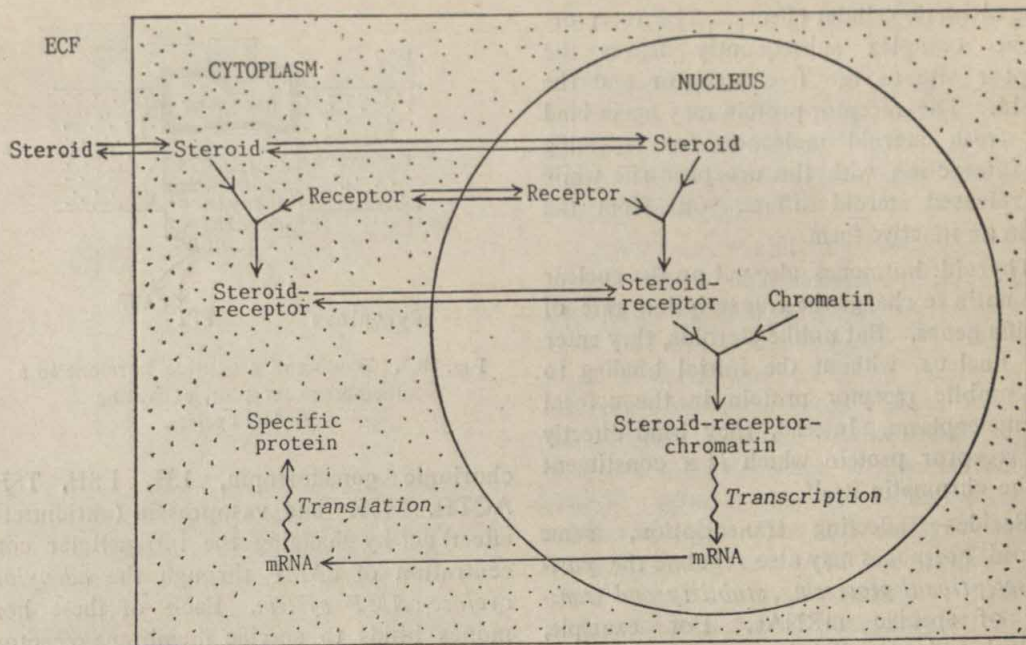


Fig. 18.1. Mechanism of action of steroid hormones through interaction with nuclear chromatin.

binds to a specific soluble, oligomeric receptor protein (*mobile receptor*) either in the cytosol or inside the nucleus (Fig. 18.1). This changes the conformation and surface charge of the receptor protein to favour its binding to the nuclear chromatin attached to the nuclear matrix. The receptor-steroid complex is translocated to the nuclear chromatin and binds to a steroid-recognizing acceptor site, called the *hormone-responsive element* (HRE), of a DNA strand on the upstream side of the promoter site (page -86) for a specific steroid-responsive gene (Fig. 18.2). This interaction either increases or decreases the transcription rate of the relevant gene by altering the efficiency of the promoter site in binding the RNA polymerase. This respectively enhances or reduces the production of the corresponding species of mRNA. For example, cortisol, testosterone

and estradiol increase the transcription of the genes for respectively tyrosine transaminase in the liver, the androgen-binding protein in seminiferous tubules and the progesterone receptor in the uterine endometrium. The consequent change in the intracellular concentration of the mRNA alters the rate of synthesis of a structural, enzymatic, carrier

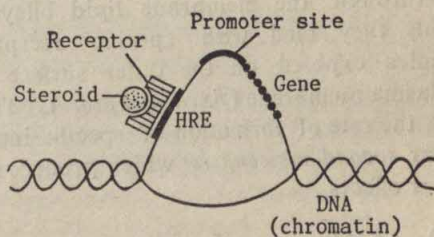


Fig. 18.2. Interaction of steroid-receptor complex and HRE on the upstream side of the promoter site of a gene.

or receptor protein coded by it. This results in the ultimate cellular effects. The receptor-steroid complex subsequently leaves the acceptor site as the free receptor and the steroid. The receptor protein may again bind with fresh steroid molecules for repeating the interaction with the acceptor site while the released steroid diffuses out from the cell in an inactive form.

Thyroid hormones also act on the nuclear chromatin to change the transcription rate of specific genes. But unlike steroids, they enter the nucleus without the initial binding to any mobile receptor protein in the cytosol or nucleoplasm. Instead, they bind directly to a receptor protein which is a constituent of the chromatin itself.

Besides affecting transcription, some steroid hormones may also regulate the *post-transcriptional processing, stability and transport* of specific mRNAs. For example, cortisol and estradiol increase the half-lives of the mRNAs coding respectively for PEP carboxykinase and the egg protein vitellogenin. Such actions may significantly change the cellular concentrations of specific mRNAs and consequently, of the corresponding proteins. Some steroids like glucocorticoids may regulate the *post-translational modifications* of specific proteins.

Interaction with membrane receptors

Hydrophilic peptide and protein hormones as well as catecholamines cannot enter target cells through the membrane lipid bilayer. Instead, they bind with specific receptor molecules exposed on the outer surface of the plasma membrane (*fixed receptors*). This alters the rate of formation of specific intracellular *second messengers* which produce the cellular effects.

(a) cAMP as second messenger :

Hormones such as glucagon, adrenaline, calcitonin, parathyroid hormone, human

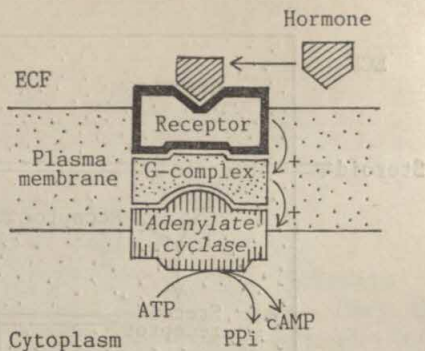


Fig. 18.3. Binding of a peptide hormone to a membrane receptor, activating adenylate cyclase.

chorionic gonadotropin, LH, FSH, TSH, ACTH, MSH and vasopressin (antidiuretic effect) act by changing the intracellular concentration of cAMP through the *adenylate cyclase-cAMP system*. Each of these hormones binds to specific membrane receptors on the outer surface of the plasma membrane (Fig. 18.3). Different types of these receptors remain associated with either G_s or G_i type of GTP-dependent, trimeric *nucleotide regulatory complexes* (N - or G -complex) of the membrane. G_s and G_i molecules bear identical β and γ subunits, but different α subunits, viz., α_s and α_i respectively. Formation of the receptor-hormone complex promotes the binding of GTP to the α subunit of either G_s or G_i followed by the release of the corresponding α -GTP complex from the latter : $\alpha\beta\gamma$ (or $\alpha\beta\gamma$ -GDP) + GTP $\rightarrow$ $\beta\gamma$ + α -GTP (+ GDP). The α_s -GTP complex, when so released from G_s , binds to *adenylate cyclase*, located on the cytoplasmic surface of the membrane, and changes its conformation to activate it. However, in some cells, calmodulin- $4Ca^{2+}$ complex is required in addition to α_s -GTP for activating adenylate cyclase. Adenylate cyclase increases the intracellular cAMP concentration by converting ATP to PPI and cAMP in the cytoplasm. Such is the course of events on the binding of

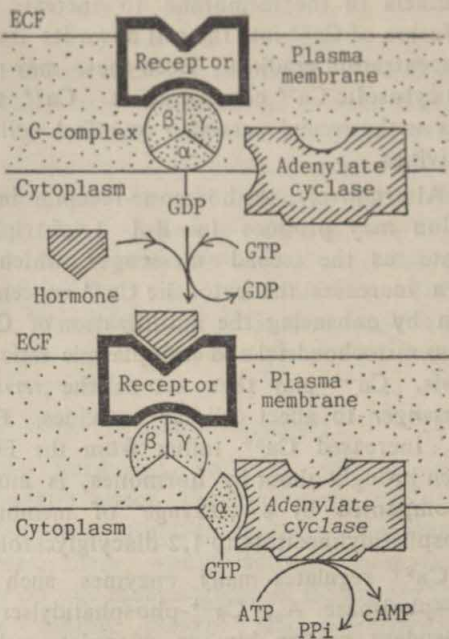


Fig. 18.4. A model for the signal transducer action of the G-complex in activating adenylate cyclase.

catecholamines to β -adrenergic receptors, of vasopressin to its receptors on renal tubule cells, or of glucagon, TSH, somatostatin, and parathormone to their respective membrane receptors. On the contrary, when α_i -GTP is similarly released from G_i , it binds to adenylate cyclase to inhibit the latter through conformational changes. This lowers the intracellular cAMP concentration. Binding of catecholamines to α_2 -adrenergic receptors in the membrane decreases the intracellular cAMP concentration in this way.

Both α_s and α_i possess GTPase activity and immediately hydrolyze the bound GTP to GDP and P_i . The α_s then reunites with β and γ subunits to reconstitute the inactive G_s . Its reactivation requires a fresh hormone molecule to bind to the membrane receptor, enabling thereby the binding of a fresh GTP molecule to the α_s subunit of G_s . In case

of α_i -GTP, the GDP produced by ATPase action continues to remain as inactive α_i -GDP which rejoins the β and γ subunits to form $\alpha_i\beta\gamma$ -GDP. The active α_i -GTP complex is released from the latter when GTP replaces GDP, following a fresh hormone-receptor binding.

cAMP acts mainly by activating some protein kinases allosterically. The cAMP-dependent protein kinases are made of allosteric (A) and catalytic (C) subunits; some of them are tetramers (A_2C_2). The catalytic subunit is inactive when bound to the allosteric subunit. cAMP binds to the allosteric subunit which is released from the catalytic subunit as the cAMP-allosteric subunit complex. The free catalytic subunit is active and uses ATP to phosphorylate specific enzymatic or structural proteins. Such cAMP-mediated phosphorylation activates enzymes such as phosphorylase kinase and triacylglycerol lipase, but inactivates other enzymes like myosin light-chain kinase and glycogen synthase.

When the cAMP level falls in the cell, the allosteric subunit of protein kinase is released from the cAMP-allosteric subunit complex and reunites with the catalytic subunit to reconstitute the inactive protein kinase. The released cAMP is subsequently inactivated through conversion to 5'-AMP by cAMP phosphodiesterase: $cAMP + H_2O \rightarrow 5'-AMP + H^+$.

(b) *Polyphosphoinositol and diacylglycerol as second messengers:*

Some hormones such as vasopressin (while acting on smooth muscles), TRH, GnRH and gastrin activate the phospholipase C-polyphosphoinositol system to produce inositol 1,4,5-triphosphate and diacylglycerol as second messengers. The hormone first binds to its specific receptor protein on the external surface of the plasma membrane. This activates a trimeric nucleotide regulatory

complex (N_p complex) of the membrane, in a manner similar to that of the adenylate cyclase system. The N_p complex in turn activates phospholipase C on the inner surface of the membrane. Phospholipase C hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) of the membrane into inositol 1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol (DG): $PIP_2 + H_2O \rightarrow IP_3 + DG$. IP_3 and diacylglycerol are released as second messengers in the cytosol and the membrane lipid matrix respectively.

Inositol 1,4,5-triphosphate enhances the mobilization of Ca^{2+} into the cytosol from intracellular Ca^{2+} pools of mitochondria and endoplasmic reticulum. Ca^{2+} then acts as the *tertiary messenger* to produce cellular effects.

Diacylglycerol activates the Ca^{2+} -phosphatidylserine-dependent protein kinase C, located on the inner surface of the membrane, by lowering its K_m for Ca^{2+} . This enzyme then phosphorylates specific enzymes and other proteins in the cytosol to modulate their activities. Protein kinase C may also phosphorylate and activate the integral membrane protein functioning in the Na^+ - H^+ antiport; this lowers the intracellular H^+ concentration and consequently changes many enzymatic reactions.

(c) Ca^{2+} as messenger :

Vasopressin (when acting on smooth muscles), dopamine, GnRH and catecholamines (α_1 effects) bind to respective membrane receptors to increase the cytosolic Ca^{2+} concentration. These receptors are, however, different from those for activating the adenylate cyclase-cAMP system.

The hormone-receptor binding may directly inhibit the Ca^{2+} -ATPase (calcium pump) and/or the Na^+ - Ca^{2+} exchanger of the membrane, both of which would otherwise extrude Ca^{2+} from the cell. It may also directly open up voltage-independent Ca^{2+}

channels in the membrane to increase the diffusion of Ca^{2+} into the cell down its inward concentration gradient. Both these may raise the cytosolic Ca^{2+} concentration. Ca^{2+} then acts as the *second messenger* to affect cellular activities.

Alternatively, the hormone-receptor interaction may produce inositol 1,4,5-triphosphate as the second messenger which in turn increases the cytosolic Ca^{2+} concentration by enhancing the mobilization of Ca^{2+} from mitochondrial and endoplasmic reticular pools. Ca^{2+} may then act as the *tertiary messenger* to affect cellular activities. Even the increased Ca^{2+} influx from the ECF, when brought about by hormones, is mostly accompanied by a cleavage of membrane phosphatidylinositols to 1,2-diacylglycerols.

Ca^{2+} regulates many enzymes such as phospholipase A_2 , Ca^{2+} -phosphatidylserine-dependent protein kinases, guanylate cyclase, adenylate cyclase and glycogen synthase. By increasing phospholipase A_2 activity, Ca^{2+} enhances the synthesis of prostaglandins. Ca^{2+} also changes membrane permeability, exocytosis, endocytosis and cell motility. Many of its effects are mediated through its binding to *Ca^{2+} -dependent regulatory proteins* like calmodulin and troponin. Calmodulin is a small, thermolabile, anionic protein (MW 16.7 kdal) of cytosol and cellular membranes; Ca^{2+} binds to its four domains to form calmodulin. $4Ca^{2+}$. This enhances the α -helix content of calmodulin by 10% to change its secondary and tertiary structures and enables it to bind to and regulate many enzymes like Ca^{2+} -ATPase, calmodulin-dependent protein kinases, myosin light-chain kinase and cAMP phosphodiesterase. Even in absence of Ca^{2+} , however, calmodulin remains tightly bound to a few enzymes; the δ subunit of glycogen phosphorylase, for example, consists of calmodulin. Binding of Ca^{2+} to the calmodulin subunit activates such enzymes. Ca^{2+} -calmodulin also regulates

many structural components like actin microfilaments. The rise in intracellular Ca^{2+} concentration and the formation of calmodulin- 4Ca^{2+} complex seem also to be involved in the LH-releasing action of GnRH. Troponin of skeletal and cardiac muscles has a TpC component resembling calmodulin in primary and secondary structures. TpC changes into active TpC- 4Ca^{2+} on binding Ca^{2+} at its four Ca^{2+} -binding sites. This enables troponin to block the inhibitory action of tropomyosin on actin-myosin interaction (page 572).

(d) *cGMP as second messenger :*

Some hormones such as insulin and somatostatin affect the *guanylate cyclase-cGMP system*. Binding to their membrane receptors, these would activate *guanylate cyclase* on the inner surface of the membrane and/or inhibit *cGMP phosphodiesterase*. These would respectively increase the guanylate cyclase-catalyzed conversion of GTP to cGMP and PPI, and decrease the phosphodiesterase-catalyzed hydrolysis of cGMP to 5'-GMP. Both would enhance the cytosolic concentration of cGMP which may activate cGMP-dependent protein kinases. These are dimeric enzymes made of two identical subunits (C_2). Their cGMP-mediated activation does not involve any dissociation of the subunits from each other : $\text{C}_2 + 2\text{cGMP} \rightarrow \text{cGMP}_2\text{C}_2$. The active cGMP-protein kinase complex (cGMP_2C_2) would in turn phosphorylate specific cellular proteins to change their activities, leading to relaxation of smooth muscles, vasodilatations and other effects. But the idea that cGMP acts as the second messenger for these hormones has not been substantiated yet. Indeed, in many cases, Ca^{2+} may act as the second messenger to activate guanylate cyclase and thereby increase the intracellular cGMP.

(e) *Phosphorylation of tyrosine kinase :*

No second messenger has been identified

for insulin, growth hormone, prolactin, oxytocin, insulin-like growth factors, epidermal growth factor (EGF) and platelet-derived growth factor (PDGF). However, binding of insulin, EGF and PDGF to their respective membrane receptors activates a specific protein kinase called *tyrosine kinase*. The latter uses ATP to phosphorylate the phenolic OH group of tyrosine in specific proteins : Tyrosine-protein + ATP $\rightarrow$ ADP + phosphotyrosine-protein. This may change the activity of tyrosine-proteins to produce some cellular effects.

18.3 HORMONE RECEPTORS

Hormone receptors are proteins present in the plasma membrane, cytosol, nucleoplasm or nuclear chromatin. The receptor molecules possess at least two distinct functional domains, one for binding the hormone by ionic or hydrophobic bonds and the other for binding or associating with *signal transducer* molecules like the *hormone-responsive element* for steroids in the DNA and the *nucleotide regulatory complexes* for peptide hormones and adrenaline in the membrane (vide 18.2). Binding of the hormone to its receptor generates a signal for initiating its biological effects ; in contrast, binding of the hormone to a carrier protein in the plasma generates no such signal for biological activities. Receptors have a *much lower concentration* in the cell in contrast to the serum concentrations of carriers and can consequently be *saturated* with hormones even at physiological concentrations of the latter. Compared to the carriers, receptors possess far higher *specificities* and *affinities* for hormones. So, they can bind to and concentrate specific hormones on the surface or in the interior of the cell even at low serum hormone titres. The noncovalent bonds joining the hormone to its receptor can dissociate readily. This *reversibility* of the hormone-receptor binding accounts for the termination of hormone

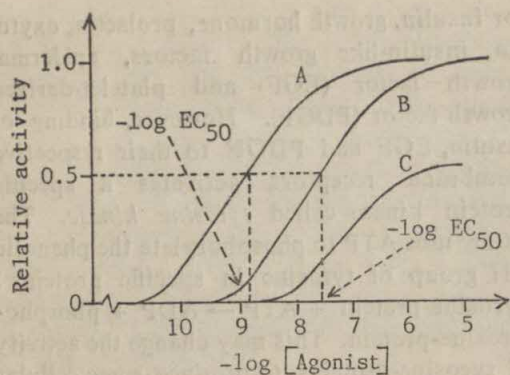


Fig. 18.5. Dose-response curves of (A) a full agonist or higher potency, (B) a full agonist of lower potency, and (C) a partial agonist.

action with the decline in the hormone titre.

Hormones and other ligands which activate specific receptors on binding to them and thereby elicit biological responses, are called *agonists*. In many cases, a minimum fraction of the receptors must get bound to the agonist for initiating a perceptible biological response (*threshold receptor occupancy*). When present in an adequate concentration, a *full agonist* activates the receptors maximally on binding to them and consequently produces the maximal biological response. However, the full agonists of a particular species of receptors may differ in their biological potency, i.e., with respect to their concentrations required for eliciting an identical maximum response (Fig. 18.5). The effective concentration of a full agonist for eliciting half the maximal biological response is called its EC_{50} and differs for different agonists of the same receptor. For example, dexamethasone, cortisol, corticosterone and aldosterone are all full agonists for the glucocorticoid receptors, but they possess a descending order of potency and an ascending order of EC_{50} , and are required in an ascending order of concentrations to elicit the maximal response by binding to those receptors. A *partial*

agonist cannot fully activate the relevant species of receptors and consequently produces only a partial biological response. Progesterone is a partial agonist for glucocorticoid receptors. Evidently, the *relative activity* of a partial agonist is less than 1 when that of a full agonist is taken as 1. A genetic disorder lowering the number or the affinity of a specific hormone receptor, or a blocking of the receptor by auto-antibodies may decrease the biological effects of the hormone to produce symptoms of its deficiency.

Antagonists are ligands which bind to the hormone receptors to antagonize or inhibit the action of agonists. For example, propranolol, a β -blocker, antagonizes the action of adrenaline on β -adrenergic receptors in the cardiovascular system while tamoxifen is an antagonist for estrogen receptors in the mammary gland. A *competitive antagonist* binds to the receptor to lower its affinity for the specific hormone and thereby increases the EC_{50} of the latter, but does not change its maximal response (Fig. 18.6). Propranolol is a competitive antagonist for β -adrenergic receptors. A *noncompetitive antagonist* lowers the maximal response to the hormone without changing its EC_{50} . A particular hormone

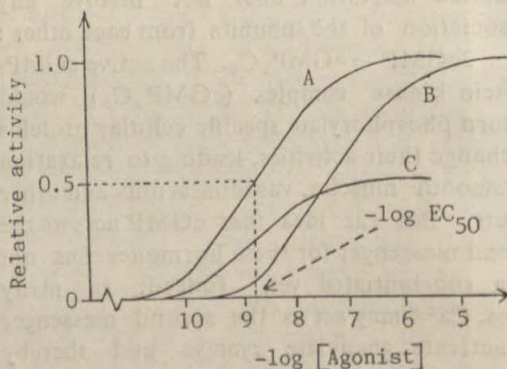


Fig. 18.6. Dose-response curves of (A) an agonist, (B) the agonist plus a competitive antagonist, and (C) the agonist plus a noncompetitive antagonist.

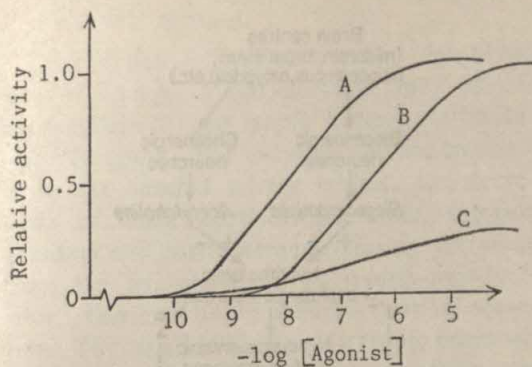


Fig. 18.7. Effects of loss of receptors on the dose-response curve of an agonist. (A) Normal receptor number. (B) Loss of some receptors when spare receptors exist. (C) Loss of some receptors in the absence of spare receptors.

may act as a full agonist, a partial agonist, an antagonist and an inactive agent for different types of hormone receptors. Androstenedione, an adrenal sexcorticoid, is an *inactive agent* for glucocorticoid receptors. *Irreversible antagonists* bind to the receptors more stably by covalent bonds and may be used in separating specific hormone receptors.

For steroids and some peptide hormones, the maximal response requires the occupancy of the full contingent of the receptors in a cell by the hormone. But catecholamines and some peptide hormones such as insulin and LH produce some responses maximally even when many of their receptors are still left unoccupied. Such unoccupied receptors are called *spare receptors* for the specific response. They *increase the sensitivity* of the cell to respond to low hormone titres, and *maintain the maximal response* inspite of a loss of some of the receptors. A hormone may leave different proportions or none of its receptors as spare ones in a tissue while maximally activating different responses; the sensitivity to a hormone is higher for a response characterized by a larger number of spare receptors. If spare receptors exist for a response, the

loss of some receptors enhances the EC_{50} of the hormone and its effective concentration for eliciting that response maximally, but leaves the maximal response itself unchanged; on the contrary, the loss of some receptors reduces the maximal response significantly if spare receptors do not exist (Fig. 18.7).

Regulation of hormone receptors

Cellular responsiveness to a hormone may be regulated by changing the number and activity of its receptors.

Regulation of receptor concentration :

Often a rise in the concentration of an agonist reduces rapidly the cellular concentration of its receptors. This *down-regulation* of the receptors decreases the sensitivity of the cell to that agonist by either enhancing the EC_{50} of the agonist or lowering the maximal response, according as spare receptors exist or not (see above). The down-regulation is brought about by a decreased synthesis or increased degradation of the receptor, or an internalization of membrane-bound receptors by pinocytosis of the membrane. For example, β -adrenergic receptors and insulin receptors are down-regulated by the rise in blood levels of respectively adrenaline and insulin. Some hormones can down-regulate the receptors for other hormones. For example, progesterone and androgens decrease the number of prolactin receptors in plasma membranes of mammary glands and liver, respectively, to antagonize the effects of prolactin on those tissues.

A few hormones (agonists) can increase the number of their own receptors in the target cells. Prolactin increases the number of prolactin receptors in the plasma membrane of mammary gland and ovary; angiotensin II increases the number of angiotensin II receptors in glomerulosa cell membrane of the adrenal cortex. Such *up-regulation* of the receptors increases the cellular responsiveness to an agonist on chronic exposure to the

latter. Some hormones can up-regulate the the receptors for other hormones. For example, thyroid hormones and glucocorticoids increase the number of β -adrenergic receptors in many tissues and of prolactin receptors in the mammary gland; estrogens enhance the number of prolactin receptors in the liver and of progesterone receptors in the uterus and mammary glands. These partly account for the synergistic actions of different hormones.

Antagonists for a receptor cannot down-regulate its number in a cell. On the contrary, a prolonged action of an antagonist may increase the number of its receptors and thereby enhance the cellular responsiveness to their agonists. For example, a chronic occupancy of some of the β -adrenergic receptors by propranolol may significantly increase their concentration in the plasma membrane.

Regulation of receptor-effector coupling :

The down-regulation of the number of receptors is often accompanied by a functional *uncoupling* of the remaining receptors from the effectors which are normally activated following the hormone-receptor binding. For example, an elevated blood adrenaline level causes a cAMP-mediated phosphorylation of β -adrenergic receptors, left behind after their down-regulation. This inactivates those receptors and prevents them from activating adenylate cyclase after binding to adrenaline. On the contrary, besides up-regulating the β -adrenergic receptors, thyroxine enhances their functional *coupling* with adenylate cyclase. This enhances the cell responsiveness to adrenaline.

18.4 REGULATION OF HORMONE SECRETION

Neuroendocrinal control

Nerve impulses control some endocrine secretions. For example, cholinergic sympathetic fibres stimulate catecholamine secretion from adrenal medulla. Nerve impulses

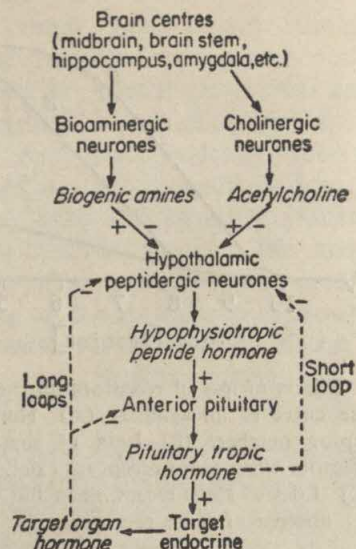


Fig. 18.8. Neuroendocrinal and feedback controls of endocrines.

from centres in the midbrain, brain stem, hippocampus, amygdala and other areas reach the hypothalamus through cholinergic and bioaminergic neurones. These neurones release respectively acetylcholine and biogenic amines (e.g., dopamine) at their terminations to regulate the secretions of hypophysiotropic peptide hormones from hypothalamic peptidergic neurones (Fig. 18.8). Nerve impulses from the uterine cervix, the vagina or the nipple are relayed through some hypothalamic neurones to the posterior pituitary to stimulate the release of oxytocin from the latter.

Some endocrine secretions are controlled by either stimulatory (*tropic*) or inhibitory hormones from a controlling gland. For example, secretions of cortical, gonadal and thyroid hormones are stimulated respectively by corticotropin, gonadotropins and thyrotropin from anterior pituitary. These tropic hormones are in turn regulated by hypophysiotropic hormones like corticotropin-releasing hormone (CRH), gonadotropin-releasing hormone (GnRH) and thyrotropin-releasing hormone (TRH) from the hypothalamus.

Feedback control

The *negative feedback* of the high blood level of a target gland hormone may inhibit the secretion of the tropic hormone stimulating that gland. For example, cortisol secreted from the adrenal cortex inhibits the secretions of both corticotropin from anterior pituitary and corticotropin-releasing hormone from the hypothalamus by a *long-loop feedback*; this results in reducing cortisol secretion. The high blood titre of a tropic hormone of anterior pituitary sometimes inhibits by a *short-loop feedback* the secretion of the corresponding hypothalamic hypophysiotropic hormone (Fig. 18.8). For example, the high blood level of corticotropin inhibits the secretion of the corticotropin-releasing hormone. A metabolite whose blood level is controlled by a hormone, may also exert a negative feedback effect on the secretion of that hormone. For example, a rise in serum Ca^{2+} from 4 to 10 mg dl⁻¹ is accompanied by a rectilinear fall in parathyroid hormone secretion while a rise in the blood glucose level inhibits glucagon secretion.

The secretion of a hormone may also be stimulated by the *positive feedback effect* of another hormone or of a metabolite. For example, the steep pre-ovulatory rise in the secretion of LH results from the stimulation of anterior pituitary by the blood estrogen level. Rise in the blood glucose level or in the serum Ca^{2+} level stimulates the secretions of respectively insulin and calcitonin.

Endocrine rhythms

Many hormones are secreted cyclically with a periodicity of 24 hours (*circadian rhythm*), more than 24 hours (*infradian rhythm*) or less than 24 hours (*ultradian rhythm*). Due to such rhythms, the peak and nadir of corticotropin secretion normally occur in the morning and around midnight respectively, growth hormone and prolactin secretions rise in the early hours of deep

sleep, and the peak of cortisol secretion is attained between 4 and 8 AM. Pituitary gonadotropins have pulsatile rhythms of secretion over the month-long menstrual cycle in women. Such endocrine rhythms may result from cyclic activities of a "biological clock" in the limbic system, supplemented by the diurnal light-dark and sleep-activity cycles and mediated by the hypothalamus.

18.5 GASTROINTESTINAL HORMONES

These peptide hormones are secreted from different parts of the gastrointestinal tract and may possess telecrine, paracrine, and neurotransmitter actions. Due to their overlapping structures and actions, major gastrointestinal hormones are grouped into two families: (a) the *gastrin family* consisting mainly of gastrin and cholecystokinin, and (b) the *secretin family* including secretin, gastric inhibitory peptide (GIP) and vasoactive intestinal peptide (VIP).

Gastrins

Gastrins are secreted by the mucosal G cells of pyloric antrum and to a smaller extent, the duodenum. Gastrin secretion is caused by the action of peptides and amino acids of gastrointestinal contents on the membrane receptors of G cells, by the stimulation of local cholinergic nerve plexuses due to gastric distention, by vagal impulses in the cephalic phase of gastric secretion, or by insulin-induced hypoglycemia. A low gastric pH (<2.5) inhibits gastrin secretion, either by directly inhibiting the G cells or by stimulating somatostatin and secretin secretions from gastrointestinal cells. [*Enterogastrones* are gastrointestinal hormones such as secretin, somatostatin, GIP and VIP, which inhibit gastric acid secretion.]

Gastrins include little gastrin (G17), large gastrin (G34) and mini gastrin (G14), made respectively of 17, 34 and 14 amino acids. All

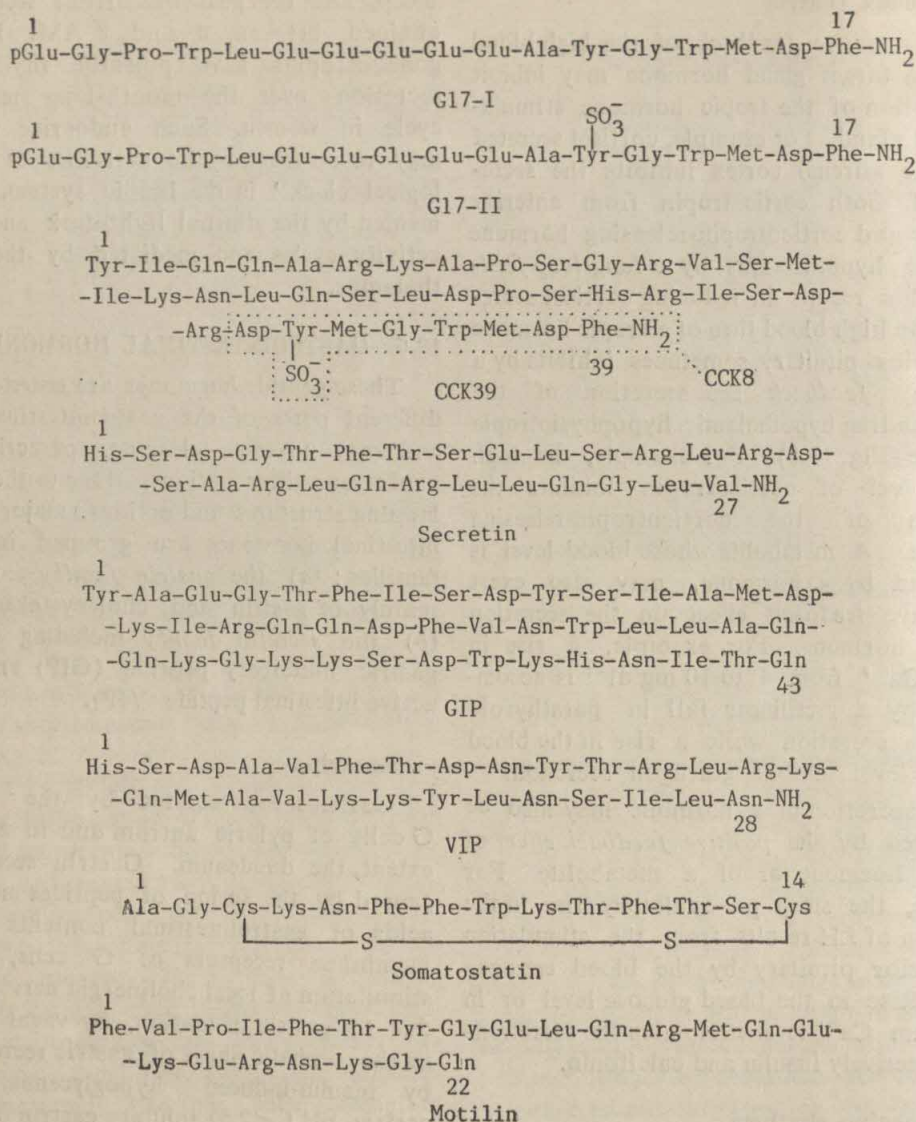


Fig. 18.9. Amino acid sequences of some gastrointestinal hormones.
pGlu: pyroglutamate.

have an identical C-terminal tetradecapeptide (C₁₄) unit considered essential for stimulating gastric acid secretion. Each exists in nonsulfated (I) and sulfated (II) forms with the tyrosine at position 12 respectively sulfate-free and sulfated (Fig. 18.9); both forms have identical potency in stimulating

gastric acid secretion. G34 quantitatively predominates in the circulation. It is probably hydrolyzed by trypsin, releasing its N-terminal part as an inactive heptadecapeptide and its C-terminal part as G17. The latter is six times as potent as G34. The N-terminal pyroglutamate (pGlu) and the

C-terminal phenylalanine amide (Phe-NH₂) residues protect G17 against respectively aminopeptidases and carboxypeptidases. Gastrins are acidic proteins having 5 consecutive Glu residues in the chain.

Functions: In pancreatic acinar cells, the binding of gastrin to the membrane receptors probably activates phospholipase C to enhance the conversion of membrane phosphatidylinositol 4,5-bisphosphates to inositol 1,4,5-trisphosphate and diacylglycerols (page 579). Either through this or by a direct action, gastrin may also enhance the intracellular Ca²⁺ concentration in these cells. Little is known about its mechanism of action elsewhere. (a) Gastrins, particularly G17, constitute the principal stimulus for the secretion of HCl by the gastric parietal cells in the gastric phase of secretion of the stomach. Gastric acid secretion may be increased even 8-fold by gastrin. The oral administration of cimetidine, an antagonist for H₂-receptors of histamine, prevents the gastrin-induced rise in gastric acid secretion; so, histamine is involved in the gastrin action. (b) Gastrins also stimulate pepsin secretion by the chief cells of stomach. Pepsin secretion consequently rises by 2-4 folds. (c) They cause vasodilatation, cell division and growth of the gastric mucosa and exocrine pancreatic tissue. (d) Gastrins enhance the frequency of the basal electrical rhythm originating in the greater curvature of the stomach and thereby increase the frequency of gastric peristalsis. (e) Gastrins enhance also the force of gastric contractions.

Zollinger-Ellison syndrome: This disease results from excessive gastrin secretion from pancreatic, duodenal or antral gastrinomas or G cell tumors. It is characterized by hypergastrinemia, gastric hyperplasia, extreme hyperchlorhydria, upper gastrointestinal ulcers, pancreatic islet tumors, watery diarrhoea and steatorrhoea. It is treated by the surgical removal of the gastrinoma

or of the entire stomach, by subcutaneous injections of a synthetic octapeptide called *minisomatostatin*, or by the oral administration of H₂-antagonists like cimetidine.

Cholecystokinins

Cholecystokinins (CCK or pancreozymins) are secreted by the I cells of duodenal and jejunal mucosa when higher fatty acids, monoacylglycerols, soaps, peptides or amino acids of intestinal contents come in contact with the microvillus membranes. Somatostatin inhibits and local cholinergic reflexes enhance CCK secretion. CCK also occurs in the gastrointestinal neurons and the brain.

CCK39, CCK33, CCK12, CCK8 and CCK4 are made of respectively 39, 33, 12, 8 or 4 amino acids. All possess the same C-terminal tetrapeptide amide as gastrins. The smaller CCK molecules have their amino acid sequences identical with those in the respective lengths of C-terminal segments of CCK39 (Fig. 18.9). The latter is thus believed to give the smaller forms by the cleaving away of different lengths of its N-terminal part. All of them occur in tissues, but CCK8 predominates in the plasma and the brain. CCK39, CCK33, CCK12 and CCK8 possess a sulfated tyrosine residue at the 7th position from the C-terminal end; this sulfate determines the full potency for stimulating gall bladder contractions and its removal considerably reduces that action, leaving CCK more gastrin-like in function.

Functions: Overlapping molecular structures enable CCK and gastrin to bind to and stimulate the receptors for each other to produce many similar biological actions. But each has a lower affinity and a higher EC₅₀ for the receptors of the other than for its own specific receptors. So, they differ in their relative activities for most biological responses. (a) The binding of CCK to the membrane receptors on pancreatic acinar

cells, enhances the intracellular concentrations of inositol 1,4,5-triphosphate, diacylglycerols and Ca^{2+} . These may act as second messengers in increasing the secretion of pancreatic enzymes such as amylase and trypsin. This is a major physiological role of CCK. (b) CCK strongly promotes the growth of pancreatic acini and gastrointestinal mucosa by enhancing the DNA content and synthesis of RNA and proteins. (c) Another major role is its stimulation of gall bladder contractions and relaxation of sphincter Oddi. This expels the bladder bile into the duodenum. (d) It amplifies the action of secretin on the secretion of HCO_3^- in the pancreatic juice and bile. (e) CCK delays gastric emptying. (f) It may be involved in regulating food intake through a stimulation of the hypothalamic satiety centre.

Secretin

Secretin is secreted mainly by the S cells of duodenal mucosa in response to the presence of acid chyme in the duodenal lumen. The secretion is initiated when the duodenal pH is lowered below 4.5. The secretion rises with the acidity of the chyme and the length of the duodenum containing the acid chyme. Secretin is secreted at the highest rate at a duodenal pH between 1 and 3, and its plasma concentration attains its peak in about 1.5 to 2 hours after a meal. The postprandial rise in plasma secretin concentration is prevented if gastric acid secretion is inhibited by the oral administration of cimetidine, an antagonist for H_2 receptors of histamine, or if the gastric chyme is evacuated from the stomach by aspiration. Pancreatic HCO_3^- , secreted under secretin action, neutralizes the acids in duodenum and thereby causes a feedback inhibition of secretin secretion.

Secretin is a linear peptide of 27 amino acids (Fig. 18.9); of its amino acid residues, 14 are common with glucagon and 9 each

with GIP and VIP. Because of such structural similarities, their biological actions show overlaps. Secretin possesses a C-terminal valinamide (Val-NH_2) protecting it against carboxypeptidases, and is a basic peptide due to the presence of 4 arginine residues and an N-terminal histidine.

Functions: (a) The principal physiological role of secretin is to stimulate the pancreatic exocrine tissue to secrete HCO_3^- and water in the pancreatic juice, an effect augmented by CCK. Probably, secretin and VIP bind as full agonists (page 582) to their respective membrane receptors on the pancreatic cells and as partial agonists to each other's receptors. The receptor-secretin binding activates adenylate cyclase to enhance the intracellular concentration of cAMP which acts as the second messenger to stimulate HCO_3^- secretion by the cells. The postprandial HCO_3^- secretion by pancreas may reach a maximal concentration of about 145 mEq l^{-1} under the stimulating action of secretin; on the contrary, it is reduced to about $\frac{1}{4}$ th of the normal on administering antisera containing anti-secretin immunoglobulins. (b) Secretin enhances protein synthesis in the pancreas and growth of the pancreatic exocrine tissue. It may also augment the stimulating action of CCK on pancreatic enzyme secretion. Unlike CCK, secretin produces these effects by generating cAMP as the second messenger instead of Ca^{2+} , inositol 1,4,5-triphosphate or diacylglycerol. (c) Secretin acts as a hydrocholeretic to stimulate secretion of HCO_3^- and water in the bile. (d) It stimulates the secretion of HCO_3^- , mucus and an epidermal growth factor from duodenal Brunner's glands. (e) Secretin inhibits gastric and duodenal motility, increases the pyloric tone, delays gastric emptying and decreases both volume and acidity of the gastric juice. These gastro-inhibitory effects result in part from its inhibitory action on gastrin secretion.

Gastric inhibitory peptide or glucose-dependent insulinotrophic peptide

This hormone (GIP) is secreted by the K cells of the duodenal and jejunal mucosa when glucose is present in the lumen of the upper small intestine. It may be secreted to a lesser extent in response to fats, peptides and amino acids in the intestinal contents.

GIP is a linear peptide made of 43 amino acids; of its amino acid residues, 15 are identical with those in glucagon and 9 with those in secretin (Fig. 18.9).

Functions: (a) The principal physiological role of GIP is the *stimulation of insulin release* in response to intraduodenal glucose, provided the blood glucose is concomitantly about 20% higher than its fasting level. This helps to metabolize the additional oral load of glucose more speedily. In the *glucose clamp technique*, an intravenous glucose infusion is continued to maintain high levels of blood glucose and plasma insulin; but the plasma GIP level shows almost no rise in spite of the constant hyperglycemia. On feeding glucose to such a hyperglycemic subject, the plasma GIP concentration rises strikingly and the plasma insulin concentration also rises in a nearly parallel time-course far above its high level attained by hyperglycemia alone. Thus, GIP is secreted in response to the intraduodenal glucose load and causes a faster and greater insulin release than after intravenous glucose infusions. GIP probably stimulates the pancreatic β cells directly to augment the stimulating action of hyperglycemia on the release of insulin. In the absence of hyperglycemia, GIP cannot enhance the release of insulin. In coeliac disease affecting the upper intestinal mucosa and consequently interfering with the secretion of GIP therefrom, poor release of insulin may produce diabetes mellitus. (b) GIP inhibits gastric acid secretion and gastric motility and consequently delays gastric emptying (*entero-*

gastrone effect). However, its enterogastrone effect seems to be less important in humans.

Vasoactive intestinal peptide

This polypeptide (VIP) occurs in the gastrointestinal tract, neurons of myenteric and submucous plexuses in the intestinal wall, and brain neurons. Stimulation of the vagus may release VIP, probably as a neurotransmitter from the intestinal plexuses. Somatostatin and its synthetic octapeptide analogue, minisomatostatin, inhibit VIP secretion.

VIP is a linear peptide of 28 amino acids, of which 9 amino acids occupy positions identical with those in secretin. It is a basic peptide due to the presence of an N-terminal histidine, 3 lysine and 2 arginine residues. It has asparagine amide (Asn-NH_2) as its C-terminal residue (Fig. 18.9).

Functions: (a) VIP relaxes vascular smooth muscles on splanchnic, salivary, pulmonary and urogenital blood vessels in particular, and causes their *vasodilatation*. VIP, released from pelvic efferents during defaecation, may cause vasodilatation and hyperemia in the colon. (b) VIP stimulates the pancreatic exocrine tissue to increase *pancreatic HCO_3^- secretion*. VIP activates the adenylate cyclase-cAMP system in the pancreatic cells by binding as full agonist to its own receptors and as partial agonist to the secretin receptors on the membrane. This generates cAMP as the second messenger which ultimately stimulates HCO_3^- secretion. (c) VIP also stimulates the *secretion of insulin and glucagon* by pancreatic islet cells. (d) It stimulates *hepatic glycogenolysis* and consequently raises the blood sugar. (e) It stimulates *lipolysis* in the adipose tissue to mobilize fatty acids in the blood. Both the glycogenolytic and lipolytic actions result from a rise in the intracellular cAMP in respectively hepatocytes and adipocytes. VIP binds with its specific membrane receptors on those cells to activate adenylate cyclase

which generates cAMP in the cells. Secretin is a partial agonist for these receptors. (f) VIP inhibits gastric acid secretion and the movements of stomach and gall bladder. During deglutition, reflexes initiated from pharynx and esophagus may release VIP from nerve endings in the stomach; VIP then favours gastric filling by relaxing the cardiac sphincter and the gastric muscles. (g) It increases the secretions and reduces the motilities of intestines. (h) It stimulates duodenal Brunner's glands to secrete HCO_3^- , mucus and an epidermal growth factor. (i) VIP may act as a neurotransmitter, particularly in the GI tract.

Verner-Morrison syndrome: This disease results from excessive VIP secretion from pancreatic or neuronal tumors. It is characterized by high plasma VIP, severe watery diarrhoea, hypochlorhydria, hypokalemia, and hypotension; frequently, hyperglycemia, hypokalemic paralysis and renal failure may occur. It is treated by the surgical removal of the tumor or by subcutaneous injections of minisomatostatin.

Somatostatin

This peptide of 14 amino acids has an intrachain S-S linkage joining the cysteine residues at the 3rd and 14th positions (Fig. 18.9). It is secreted by the D cells of the antral and duodenal mucosa and pancreatic islets when digestion products of proteins, carbohydrates and fats occur in the gastrointestinal lumen or when the gastric acid chyme enters the duodenal bulb. It is secreted from the hypothalamus also. In contrast to the telecrine action of hypothalamic somatostatin on anterior pituitary, the gastrointestinal somatostatin has local paracrine actions limited to the gastrointestinal mucosa and pancreas.

Functions: (a) Pancreatic somatostatin inhibits the secretions of insulin and glucagon from pancreatic islets. (b) It may also directly inhibit secretions of both HCO_3^- and

enzymes in the pancreatic juice. (c) Gastrointestinal somatostatin inhibits the secretions of gastrin, secretin, CCK, GIP and motilin. (d) It also inhibits some of their actions such as gastric acid secretion, secretion of Brunner's glands, pancreatic HCO_3^- and enzyme secretions, gastric emptying and gall bladder contractions.

Motilin

This linear peptide of 22 amino acids is secreted by enterochromaffin cells of duodenal and jejunal mucosa (Fig. 18.9). Its secretion continues even on fasting, rises only slightly after food intake and is inhibited by intravenous glucose infusion or somatostatin.

Functions: Motilin enhances myoelectric activities and contractions of gastrointestinal muscles. Rhythmic fluctuations in plasma motilin fluctuates gastrointestinal motility in the interdigestive period. In the fasting state, motilin-induced gastrointestinal movements may prevent bacterial growth in the intestinal lumen by freeing it from debris and secretions. After food intake, gastric emptying is promoted by motilin-induced gastric movements. Motilin simultaneously contracts the cardiac sphincter to prevent the regurgitation of acid chyme into the esophagus.

Enteroglucagon

Enteroglucagon or glucagon-like immunoreactivity (GLI) includes one or more peptides (e.g., glicentine from porcine intestine) which immunologically resemble glucagon, but are not chemically identical with the latter. Enteroglucagon is secreted from gastroduodenal A cells and ileocolonic L cells, when glucose or fat enters the gastrointestinal lumen.

(a) Enteroglucagon, like glucagon, stimulates hepatic glycogenolysis, but not strongly enough to cause hyperglycemia. It may stimulate (b) lipolysis in the adipose tissue and (c) the growth of the small intestinal mucosa. Prolonged fasting lowers entero-

glucagon secretion and causes an atrophy of intestinal mucosa ; high food intake increases enteroglucagon secretion and also the growth of the intestinal mucosa. (d) Enteroglucagon inhibits the secretions of gastrin, gastric HCl and pancreatic juice.

18.6 HYPOTHALAMIC HORMONES

Hypothalamus secretes several peptide hormones which regulate the secretions of the anterior pituitary hormones. These *hypophysiotropic hormones* include thyrotropin releasing hormone (TRH), corticotropin releasing hormone (CRH), gonadotropin releasing hormone (GnRH), growth hormone releasing hormone (GHRH), somatostatin, prolactin release-inhibiting hormones (PRIH) and vasoactive intestinal peptide (VIP). They are synthesized by hypothalamic *peptidergic neurons* constituting the *parvicellular neurosecretory system* in the lateral wall of the 3rd ventricle. Axons of these neurons store the hormones in small dense vesicles (diam. 80-120 nm) at their terminals and end close to the capillaries of the *hypophyseal portal system* in the median eminence. The parvicellular neurons release their hormones into the hypophyseal portal vessels which carry them to the anterior pituitary.

Regulation

The basic autonomy of hypothalamic peptidergic neurons is indicated by the absence of significant changes in the basal secretions of the growth hormone and gonadotropins, and in the stress-induced secretion of corticotropin (ACTH) from the anterior pituitary even after the surgical isolation of the hypothalamo-hypophysis complex from extrinsic neuronal connections.

Normally, however, secretions of the hypothalamic hormones are considerably modulated by neurons reaching the parvicellular system from different brain areas such as the midbrain, brain stem, hippocampus,

amygdala, anterior thalamus, septal nuclei, pyriform cortex and different parts of hypothalamus itself. These regulatory neurons form synapses on the cell-bodies and axons of the hypothalamic peptidergic neurons and release at their synaptic ends either biogenic amines like noradrenaline, dopamine and serotonin or acetylcholine ; these either stimulate or inhibit the release of hormones from the peptidergic neurons (Fig. 18.8). For example, impulses through noradrenergic neurons stimulate the release of GHRH and TRH during stress ; serotonergic neurons may increase the release of CRH and GHRH, but inhibit the release of TRH ; CRH is released due to nerve impulses reaching the limbic system owing to emotions, psychic stimuli, hypoglycemia and trauma ; but CRH release is inhibited by impulses from noradrenergic neurons.

Rise in the blood level of a target gland hormone often regulates the release of the hypothalamic hormone by a *long-loop feedback effect* (Fig. 18.8). For example, a high blood cortisol level inhibits the release of CRH ; somatomedin C, a mediator for some actions of growth hormone, inhibits the release of GHRH and stimulates that of somatostatin ; a high blood level of either testosterone or estradiol inhibits the release of GnRH. Rise in the blood titre of some pituitary hormones may also inhibit the secretion of respective hypophysiotropic hormones by *short-loop feedback effects*. For example, enhanced blood levels of corticotropin, gonadotropins and GH inhibit the release of respectively CRH, GnRH and GHRH.

Many hypophysiotropic hormones such as CRH and GnRH are secreted in pulsatile manners and in specific biological rhythms.

Structures and actions

In general, hypophysiotropic hormones enhance or reduce the secretions of anterior

pituitary hormones by respectively increasing or decreasing the cytoplasmic Ca^{2+} concentration in the pituitary cells. But they differ in their mechanism for changing the Ca^{2+} concentration and are accordingly placed in three groups.

(a) *Hormones activating adenylate cyclase :*

CRH, isolated from sheep hypothalamus, is a peptide of 41 amino acids (Fig. 18.10). It stimulates the release of corticotropin from corticotroph cells of anterior pituitary, but probably not its synthesis. It may also stimulate the release of β -lipotropin from the anterior lobe.

GHRH or *somatocrinin* is a peptide made of 44 amino acids. *GHRH* increases the synthesis of GH by stimulating the transcription of the GH gene in somatotroph α -cells of anterior pituitary. It also stimulates the release of GH.

VIP is a basic peptide of 28 amino acids. It has an N-terminal histidine, a C-terminal asparagine amide, 3 lysine and 2 arginine residues, and is identical with the gastrointestinal VIP (Fig. 18.9). It stimulates the lactotroph and corticotroph cells of anterior

pituitary to secrete respectively prolactin and corticotropin.

When these hormones bind to their membrane receptors on the respective target cells in anterior pituitary, *adenylate cyclase* gets activated and catalyzes the conversion of ATP to cAMP in the cytoplasm (page 578). cAMP then acts as the second messenger on the membrane Ca^{2+} channels to enhance Ca^{2+} influx. The consequent increase in the intracellular Ca^{2+} concentration stimulates the secretion of the respective pituitary hormones.

(b) *Hormones activating phospholipase C :*

TRH is a neutral tripeptide with pyroglutamate and prolinamide as respectively the N-terminal and C-terminal amino acids (Fig. 18.10). It stimulates the transcription of the thyrotropin gene in pituitary thyrotroph cells to increase the synthesis of thyrotropin. It also enhances the release of thyrotropin from them. It stimulates also the transcription of the prolactin gene and the release of prolactin from pituitary lactotroph cells. Blood TRH rises in hyperthyroidism resulting from hypothalamic hyperfunction.

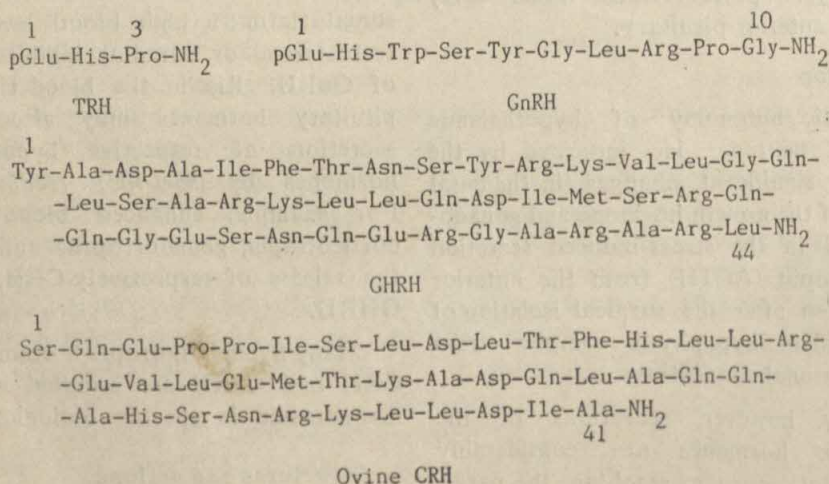


Fig. 18.10. Amino acid sequences of some hypothalamic hormones.
pGlu : pyroglutamate.

GnRH or *LHRH* is a decapeptide with pyroglutamate and glycine as respectively the N-terminal and C-terminal amino acids (Fig. 18.10). It stimulates the pituitary gonadotroph cells to increase synthesis as well as secretion of both the pituitary gonadotrophins, FSH and LH. A high blood level of estrogens sensitizes the pituitary gonadotrophs to the action of *GnRH*.

On binding to their membrane receptors on respectively thyrotroph and gonadotroph β -cells, TRH and *GnRH* activate *phospholipase C* in the membrane. Phospholipase C catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-triphosphate and diacylglycerol which act as second messengers to increase the intracellular Ca^{2+} concentration (pages 579-580). This enhances the secretions of the respective pituitary hormones. Simultaneously, protein kinase C, activated by Ca^{2+} and diacylglycerol, activates adenylate cyclase which increases cAMP in the cells; this further increases the intracellular Ca^{2+} concentration to sustain the secretory response.

GAP (*GnRH*-associated peptide) is made of 56 amino acids, first ten of which are similar to *GnRH*. It mimics the gonadotropin releasing action of *GnRH* and inhibits the release of prolactin from the anterior lobe. But its physiological role and mechanism of action are uncertain.

(c) *Hormones lowering intracellular Ca^{2+} concentration*:

Somatostatin is a tetradecapeptide with an intrachain S-S linkage involving the C-terminal cysteine residue (Fig. 18.9). It is also secreted from gastrointestinal mucosa and pancreas (page 590). The hypothalamic somatostatin inhibits the release of growth hormone from pituitary somatotroph cells by lowering the intracellular Ca^{2+} concentration. It may inhibit Ca^{2+} influx into those cells either by directly closing [membrane] Ca^{2+}

channels, or indirectly through a reduction in K^+ outflow, or by decreasing the translocation of Ca^{2+} into the cytosol from Ca^{2+} pools of endoplasmic reticulum and mitochondria. Somatostatin can also inhibit the TRH-mediated release of thyrotropin.

Dopamine (Fig. 12.22) is released from some hypothalamic neurons. It acts as a prolactin release inhibiting hormone. It inhibits the transcription of prolactin gene in pituitary lactotroph cells and consequently decreases prolactin synthesis. It also inhibits the release of prolactin from the anterior lobe. Dopamine acts by lowering the intracellular Ca^{2+} concentration in some way. This action may partly result from an inactivation of adenylate cyclase through the inhibitory G_i complex of the membrane, following the binding of dopamine to membrane receptors.

18.7 POSTERIOR PITUITARY HORMONES

Human posterior lobe hormones are two nonapeptides, *vasopressin* (arginine vasopressin) and *oxytocin*, differing only in their 3rd and 8th amino acid residues (Fig. 18.11). Their biological activities depend upon the C-terminal glycine amide, the sidechain amide

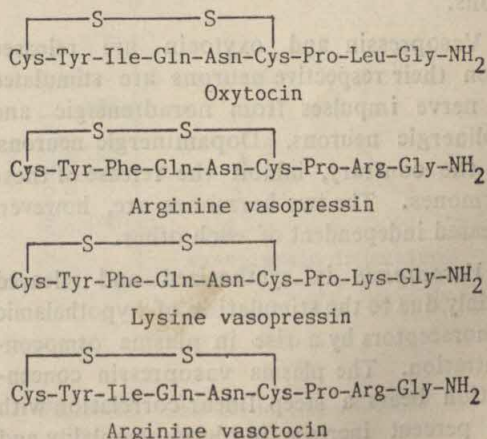


Fig. 18.11. Amino acid sequences of posterior lobe hormones.

groups of glutamine and asparagine, the hydroxyphenyl group of tyrosine, and the intrachain S-S linkage between cysteine residues at the 1st and 6th positions. Their close structural similarities result in considerable overlapping of their biological actions.

Secretion and regulation

Oxytocin and vasopressin are synthesized by magnocellular neurons of respectively paraventricular and supraoptic nuclei of hypothalamus in the form of two species of inactive precursor proteins. Membrane-bound neurosecretory vesicles containing these precursor proteins descend at the rate of about $2 \mu\text{m sec}^{-1}$ along the axons of these neurons and remain stored in their expanded axon terminals near blood vessels of the posterior pituitary. When these neurosecretory neurons are stimulated, action potentials flow to their axon terminals to cause proteolysis of the precursor molecules into either oxytocin and inactive *neurophysin I* (MW 19 kdal), or vasopressin and inactive *neurophysin II* (21 kdal). These are released from the axon terminals and pass into the blood. Neurophysins I and II thus act in effect as carrier proteins for respectively oxytocin and vasopressin in the neurosecretory axons.

Vasopressin and oxytocin are released when their respective neurons are stimulated by nerve impulses from noradrenergic and cholinergic neurons. Dopaminergic neurons, on the contrary, inhibit the release of these hormones. The two hormones are, however, released independent of each other.

Vasopressin is synthesized and released mainly due to the stimulation of hypothalamic osmoreceptors by a rise in plasma osmoconcentration. The plasma vasopressin concentration bears a steep linear correlation with the percent increase in blood osmolality and rises considerably due to even small rises in blood osmolality (Fig. 18.12). Hypertonic

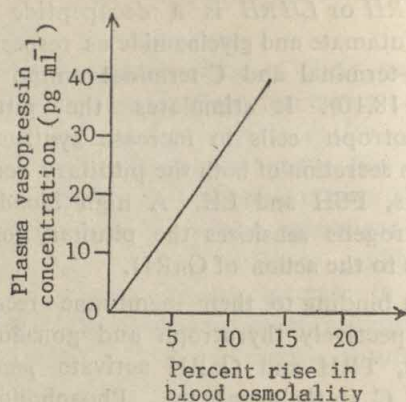


Fig. 18.12. Relation between plasma vasopressin concentration and percent rise in blood osmolality.

saline infusions increase the rate of vasopressin release even when they raise the plasma osmolality by just 2%; the fall in plasma osmolality due to hemodilution reduces the rate of vasopressin release.

In addition, vasopressin synthesis and release may be enhanced by nerve impulses from the left atrial volume receptors, stimulated by a fall in the blood volume as in case of a hemorrhage. Adrenaline and ethanol inhibit vasopressin release by expanding the blood volume. Vasopressin release may also be augmented by nerve impulses from the carotid and aortic baroreceptors owing to hypotension resulting from the decline in blood volume. However, the plasma vasopressin concentration is a curvilinear function of the percent fall in blood volume and rises but little for small changes in the latter (Fig. 18.13)—vasopressin release requires at least a 8% fall in the blood volume.

Vasopressin is also released in response to angiotensin II.

Oxytocin is released primarily by reflexes due to nerve impulses from touch receptors in the nipple during suckling by the child. This explains the uterine contractions recorded during suckling. Even conditioned stimuli

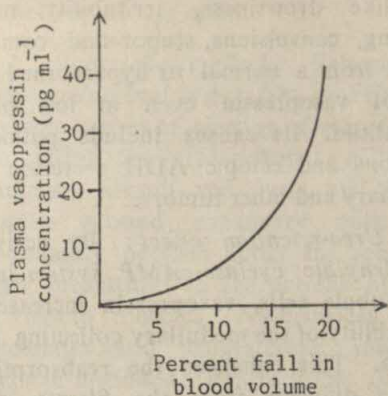


Fig. 18.13. Relation between plasma vasopressin concentration and percent fall in blood volume

associated with suckling may stimulate oxytocin release; e.g., the sight and sound of the infant can evoke oxytocin-mediated ejection of milk. Oxytocin is also released during coitus due to reflexes initiated by stimulations of the female external genitalia. During parturition, oxytocin-releasing reflexes result from the stimulation of cervical stretch receptors by the dilatation of cervix by full-term fetus. Even inflation of a balloon inserted in the vagina produces strong uterine contractions through the reflex secretion of oxytocin (*Ferguson reflex*). During parturition, the rise in oxytocin secretion is further helped by a rise in the synthesis of oxytocin-neurophysin I precursor protein, brought about by a fall in the blood level of progesterone and the consequent removal of its inhibitory effect. Estrogens increase the synthesis of this precursor protein.

Actions of vasopressin

1. *Antidiuretic effect*: Vasopressin, also called the *antidiuretic hormone* (ADH), increases the reabsorption of urinary water in the distal tubules and collecting ducts of mammalian and avian kidneys. This effect is mediated through the stimulation of the *adenylate cyclase-cAMP* system by vaso-

pressin. Released due to the rise in plasma osmolality, vasopressin goes to bind with specific receptors on the basal and lateral plasma membrane of the distal tubule and collecting duct cells. This activates adenylate cyclase of the membrane (page 578). cAMP produced by the adenylate cyclase action flows through the cytoplasm to the luminal membrane of the cell to activate a membrane-bound protein kinase there. The latter uses ATP to phosphorylate specific proteins of the luminal membrane and thereby increases the permeability of the luminal membrane to water. This enhances the osmotic reabsorption of water from the tubular lumen to the surrounding hypertonic medullary inter-

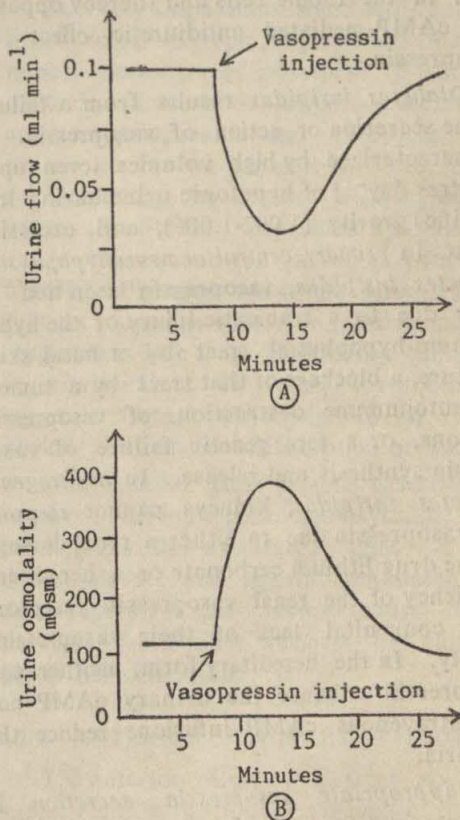


Fig. 18.14. Changes in (A) urine flow and (B) urine osmolality on vasopressin injection.

stitium. Vasopressin consequently causes the formation of hypertonic urine, having low volume, high-specific gravity and high concentrations of Na^+ , Cl^- , H_2PO_4^- and urea (Fig. 18.14). The antidiuretic effect is accompanied by an aggregation of some membrane proteins, seen in freeze-fracture replicas of the membrane. Halothane, but not phloretin, inhibits the vasopressin-induced antidiuretic effect; it is also inhibited by colchicine, vinblastine and other inhibitors for microfilaments and microtubules.

The antidiuretic action of vasopressin is modulated by prostaglandins. Vasopressin stimulates prostaglandin synthesis in the kidney; PGE_2 in turn decreases the cAMP level in the tubule cells and thereby opposes the cAMP-mediated antidiuretic effect of vasopressin.

Diabetes insipidus results from a failure in the secretion or action of vasopressin. It is characterized by high volumes (even upto $20 \text{ litres day}^{-1}$) of hypotonic urine having low specific gravity (1.002-1.006), and excessive thirst. In *primary, central or neurohypophysial diabetes insipidus*, vasopressin secretion is poor due to a traumatic injury of the hypothalamo-hypophysial tract by a basal skull fracture, a blockage of that tract by a tumor, an autoimmune destruction of vasopressin neurons, or a rare genetic failure of vasopressin synthesis and release. In *nephrogenic diabetes insipidus*, kidneys cannot respond to vasopressin due to either a renal damage by the drug lithium carbonate or a hereditary deficiency of the renal vasopressin receptors or a congenital lack of their vasopressin-affinity. In the hereditary form, neither can vasopressin enhance the urinary cAMP nor can intravenous cAMP infusions reduce the polyuria.

Inappropriate vasopressin secretion is characterized by a persistently hypertonic urine, progressive renal loss of Na^+ , low serum Na^+ , and symptoms of water intoxica-

tion like drowsiness, irritability, nausea, vomiting, convulsions, stupor and coma. It results from a normal or hypernormal secretion of vasopressin even at low plasma osmolalities. Its causes include pulmonary infections and ectopic ADH secretion from pulmonary and other tumors.

2. *Urea-retention effect*: By activating the *adenylate cyclase-cAMP* system in the renal tubule cells, vasopressin increases the permeability of the medullary collecting ducts to urea. This enhances the reabsorption of urea by diffusion from the filtrate in the collecting ducts. The reabsorbed urea contributes to the hypertonicity of the medullary interstitium and consequently, to the antidiuretic effect of vasopressin. Urea-retention effect is inhibited specifically by phloretin, but not by halothane, and consequently appears to involve a cAMP-dependent mechanism distinct from that for the antidiuretic effect.

3. *Pressor effect*: Secreted due to a critical fall in the volume or pressure of blood, vasopressin stimulates the contraction of smooth muscles and thereby causes peripheral arteriolar vasoconstriction. This raises the peripheral resistance and arterial blood pressure. Vasopressin causes smooth muscle contractions by increasing *cytosolic Ca^{2+} concentration*. On binding to membrane receptors on smooth muscle cells, vasopressin activates phospholipase C which generates inositol 1,4,5-triphosphate and diacylglycerol. These in turn increase the cytosolic Ca^{2+} concentration by enhancing Ca^{2+} influx from ECF, and Ca^{2+} translocation from organelles to the cytosol. Ca^{2+} joins calmodulin to form the calmodulin- 4Ca^{2+} complex which stimulates smooth muscles.

4. *Glycogenolytic effect*: Vasopressin stimulates glycogenolysis by increasing the cytosolic Ca^{2+} concentration in hepatocytes.

The antidiuretic action is the major physiological role of vasopressin.

Actions of oxytocin

1. *Galactobolic effect*: Released due to a *neuroendocrinal reflex* (the suckling reflex), oxytocin causes an ejection of stored milk by contracting the myoepithelial cells around mammary alveoli and ducts, and the smooth muscles around mammary milk sinuses. Uncertainty persists about the second messenger mediating in the actions of oxytocin on smooth muscles. Nevertheless, estradiol potentiates and progesterone inhibits the oxytocin-mediated contractions of smooth muscles. Estrogens progressively increase the number of oxytocin receptors on the plasma membrane of myoepithelial and smooth muscle cells of the mammary gland during pregnancy. Progesterone decreases the number of oxytocin receptors and also inhibits the secretion of oxytocin. With the fall in blood progesterone level near the end of pregnancy, these inhibitory effects of progesterone are removed. Consequently, the density of mammary oxytocin receptors reaches a peak at parturition and makes the myoepithelial and smooth muscle cells maximally sensitive to oxytocin. Simultaneously, the suckling reflex evokes oxytocin secretion. These enable the myoepithelial and smooth muscle cells to contract for milk ejection.

2. *Uterine effect*: Secreted at full-term of pregnancy, oxytocin causes contractions of uterine muscles for child-birth (*oxytocic action*). Estrogens enhance and progesterone decreases the density of oxytocin receptors on uterine smooth muscle cells. But unlike the mammary gland, the uterine receptors remain fairly constant in number during pregnancy; they increase sharply and abruptly in number with the decline in the blood progesterone level at full-term. This enhances the sensitivity of uterine muscles to oxytocin. Simultaneously, oxytocin is secreted due to the reflex initiated by dilatation of the uterine cervix by the full-term

fetus. This triggers the uterine contractions for parturition.

Oxytocin, secreted during coitus, promotes the aspiration of semen into the uterus by contracting the uterine muscles. This is also augmented by the rise in the density of uterine oxytocin receptors due to estrogen secreted in the follicular phase of menstrual cycle.

Comparative aspects

Most mammals including primates, ruminants, Equidae and carnivores secrete arginine vasopressin and oxytocin. But pig and hippopotamus secrete *lysine vasopressin* and oxytocin (Fig. 18.11); some Suiformes secrete oxytocin and both types of vasopressin.

Reptiles, amphibians, some birds and lung-fishes secrete oxytocin, *arginine vasotocin* and minor amounts of *mesotocin* (8-isoleucine-oxytocin). Some birds like chicks secrete both arginine vasopressin and vasotocin. Vasopressin exerts its antidiuretic action in birds by increasing the tubular reabsorption of water. Vasotocin may cause vasodilatation and a consequent fall in blood pressure in lizards, alligators and some birds. It also reduces the urine volume in reptiles and many amphibians like frogs by causing glomerular vasoconstriction. In frogs, vasotocin also increases water reabsorption in the urinary bladder and cutaneous absorption of water.

Many teleost fishes secrete vasotocin and *isotocin* (4-serine-8-isoleucine-oxytocin). Vasotocin lacks the antidiuretic effect in fresh-water teleosts and aquatic amphibians like salamanders; it may instead cause diuresis in some fresh-water teleosts by enhancing glomerular filtration.

18.8 ANTERIOR PITUITARY HORMONES

The anterior pituitary secretes six recognized peptide hormones. Because of similarities in structure, action and synthesis, they are classified into three families along with some hormones from other sources.

(a) The *glycoprotein hormone family* consists of thyrotropin (TSH) and two gonadotropins, viz., follicle stimulating hormone (FSH) and luteinizing hormone (LH), all three secreted from anterior pituitary, and human chorionic gonadotropin (hCG) from the placenta. (b) The *growth hormone-prolactin-chorionic somatomammotropin family* includes two anterior lobe hormones, viz., growth hormone (GH) and prolactin (PRL), and a placental hormone called chorionic somatomammotropin. (c) The *pro-opiomelanocortin family* includes corticotropin (ACTH) of the anterior lobe, melanocyte stimulating hormone (MSH) of the intermediate lobe of pituitary, and lipotropins, endorphins and enkephalins of the pituitary and the brain.

Thyrotropin

Structure: Thyrotropin or thyroid stimulating hormone (TSH) is a dimeric glycoprotein (MW 28 kdal) secreted by thyrotroph β cells of the anterior lobe. It is made of two peptide chains, α and β , linked noncovalently. The α chain is identical for TSH, FSH and LH in the same species and consists of 92 amino acid residues in humans. The β chains are different in the three hormones, though showing considerable similarities in their amino acid sequences. The β chain of human TSH has 112 amino acid residues. Both α and β chains have several intrachain S-S linkages between their cysteine residues. Human TSH contains about 21% carbohydrate. Its α and β chains bear respectively two and one oligosaccharide chains, linked by N-glycosidic linkages to specific asparagine residues.

Synthesis: The large precursors of α and β chains are coded by distinct genes and synthesized separately. They undergo post-translational modifications and glycosylations separately, yielding the respective peptide chains which are then linked noncovalently to form the final hormone molecule.

Actions: Specific glycoprotein receptors on the thyroid cell membrane bind to the

receptor-binding site on the β chain of TSH molecules. However, specific domains of both α and β chains participate in recognizing the receptor. Thus, the β chain of the hormone endows it with the specificity of acting on thyroid cells. The receptor-TSH binding activates adenylate cyclase which catalyzes the formation of cAMP. cAMP acts as the second messenger for most TSH actions.

(a) TSH stimulates the *synthesis of thyroid hormones*. It takes only minutes to increase all phases of the biosynthesis such as the active iodide uptake, iodination of tyrosine residues of thyroglobulin, and coupling of iodotyrosines into T_4 and T_3 . It increases both the iodine concentration and the quantity of stored T_4 in the thyroid.

(b) TSH stimulates the *release of stored thyroid hormones* from the gland. Within minutes of TSH injection, endocytotic uptake of colloid droplets, proteolysis of endocytosed thyroglobulin, activity of iodotyrosine deiodinase and concentrations of T_4 and T_3 in the thyroid vein serum rise markedly.

Hypophysectomy sharply decreases the thyroidal uptake of iodides, the coupling of iodotyrosines and the serum T_4 and T_3 levels. Iodide uptake, T_4 synthesis and proteolysis of thyroglobulin are all enhanced in thyroid slices treated with either TSH or cAMP.

(c) Action of TSH over days increases the DNA content, transcription of RNA, translation of proteins, cell size and mitosis in the thyroid to cause the *growth of thyroid tissue*.

(d) Other effects on the thyroid cell include stimulations of *glycolysis, TCA cycle, pentose phosphate pathway and phospholipid synthesis*. Stimulation of the last two pathways does not involve cAMP. Phospholipid synthesis under TSH action may help to replenish the membrane removed during endocytosis of colloid droplets.

(e) TSH-induced rise in cAMP may *activate adipose tissue lipase* in adipocytes to

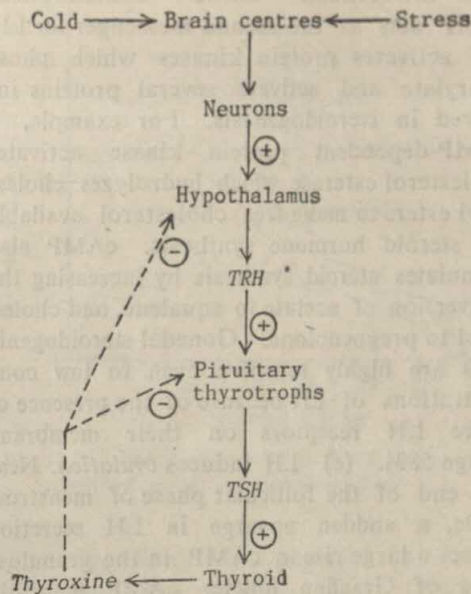


Fig. 18.15. Regulation of secretions of TSH and thyroxine.

enhance the release of fatty acids from the stored fats into the blood.

Regulation: Hypophysectomy results in thyroidal atrophy and hypothyroidism even if the pituitary is transplanted beneath the renal capsule, but not if the pituitary is transplanted in the median eminence close to the hypophyseal portal system. Electrical stimulation of hypothalamus between its preoptic nucleus and median eminence decreases the pituitary TSH content and raises the blood TSH level. These indicate the importance of hypothalamus in stimulating TSH secretion.

The basal secretion of TSH is maintained largely by the tonic action of the *thyrotropin releasing hormone* (TRH) secreted by the hypothalamus. Cold increases TSH secretion by stimulating the release of TRH. *Somato-statin* secreted by hypothalamus counteracts TRH and inhibits the release of TSH.

A rise in the blood level of thyroid hormones decreases TSH secretion by inhibiting the release of TRH from hypothalamic neurons and by reducing the sensitivity of pituitary thyrotrophs to TRH (*long-loop feedback inhibitions*) (Fig. 18.15). Administration of thiouracil lowers the blood T_4 level, leading to thyroid hypertrophy; electrolytic lesions in the anterior hypothalamus prevents such thyroid enlargement. Evidently, the thiouracil-induced fall in blood T_4 increases TSH secretion by removing the feedback inhibition of TRH and TSH by T_4 .

The blood TSH level normally averages less than 50 mg dl^{-1} in humans. It rises to the peak during sleep before midnight and declines progressively after several hours to reach the nadir before midday.

Pituitary gonadotrophins

Structure: Follicle stimulating hormone (FSH) and luteinizing hormone (LH) are glycoproteins, having MWs of about 34 and 28.5 kdal respectively and containing about 16% carbohydrate. Each is a dimer of α and β chains, linked noncovalently. The α chain is identical for TSH, FSH and LH of the same species (page 598). The β chain of human FSH and LH have respectively 118 and 112 amino acid residues and respectively two and one asparagine-linked oligosaccharide chains. Each peptide chain has several intrachain S-S linkages.

Synthesis: The large precursor molecules of α and β chains are synthesized separately in pituitary gonadotroph β cells. Post-translational modifications and glycosylations yield the α and β chains.

Actions of FSH: FSH binds to specific receptors in the plasma membranes of the granulosa cells of ovarian follicles and the Sertoli cells of testicular seminiferous tubules. This activates the *adenylate cyclase-cAMP system* to raise the intracellular cAMP concentration in those cells. cAMP acts as the

second messenger for FSH to produce the biological effects. (a) FSH stimulates the growth of Graafian follicles in the ovary by promoting the proliferation and development of the granulosa cells. This results from the cAMP-induced rise in RNA transcription and protein synthesis in follicular cells. Estrogens enhance the action of FSH on follicular cells, probably by increasing the number of FSH receptors on them. (b) FSH *up-regulates* the LH receptors, i.e., increases their number in the plasma membrane of the granulosa cells to enhance the sensitivity to the action of LH, which is to follow shortly. Thus, FSH augments the subsequent action of LH on the ovarian follicle in inducing estrogen secretion as well as ovulation. (c) The FSH-induced rise in cAMP stimulates the ovarian follicular cells to synthesize and release a serine protease called *plasminogen activator* which initiates ovulation (see below). (d) FSH-induced rise in cAMP activates protein kinases in testicular Sertoli cells. This enhances transcription of RNA and consequently, the translation of specific proteins in the Sertoli cells. FSH thus stimulates the formation of an *androgen-binding protein* by the Sertoli cells. Released by those cells into the lumen of seminiferous tubules, this glycoprotein binds to testosterone coming from Leydig cells of testis and carries it at a high concentration to the spermatogenic cells of the seminiferous tubule. This enables testosterone to stimulate growth of seminiferous tubules and spermatogenesis. Mainly in this way, FSH promotes growth of testis as well as spermatogenesis.

Actions of LH : LH, also called the interstitial cell stimulating hormone or ICSH, stimulates (a) the *synthesis of testosterone* in testicular Leydig cells and (b) the *synthesis of progesterone* in the ovarian corpus luteum. It binds to specific receptors on the plasma membranes of these cells. This activates the *adenylate cyclase-cAMP system* to raise

the intracellular cAMP concentration. cAMP acts as the second messenger for LH and activates protein kinases which phosphorylate and activate several proteins involved in steroidogenesis. For example, a cAMP-dependent protein kinase activates cholesterol esterase which hydrolyzes cholesteryl esters to make free cholesterol available for steroid hormone synthesis. cAMP also stimulates steroid synthesis by increasing the conversion of acetate to squalene, and cholesterol to pregnenolone. Gonadal steroidogenic cells are highly sensitive even to low concentrations of LH because of the presence of spare LH receptors on their membrane (page 583). (c) LH induces *ovulation*. Near the end of the follicular phase of menstrual cycle, a sudden upsurge in LH secretion causes a large rise in cAMP in the granulosa cells of Graafian follicle. cAMP manifold increases the synthesis and release of plasminogen activator by the granulosa cells. Plasminogen activator hydrolyzes an inactive β -globulin called *plasminogen* (MW 90 kdal) into an active serine protease called *plasmin* in the follicular fluid. Plasmin initiates the degradation of the follicular wall, leading to ovulation. Unlike the steroidogenic cells, the granulosa cells possess no spare receptor for LH. (d) After ovulation, the continued action of LH on the granulosa cells of the ruptured follicle initiates the synthesis of progesterone in those cells and changes the ruptured follicle into the corpus luteum (*luteinization*). (e) Prolonged action of LH *down-regulates the LH receptors*, i.e., lowers their number on corpus luteum cells, by enhancing the endocytotic internalization and degradation of those receptors. This reduces the LH sensitivity of the cells and decreases their steroidogenic response to LH.

Regulation : Gonadotropin secretions rise considerably at puberty in both sexes. In the adult male, their secretions follow a more or less continuous pattern. But in ovulating

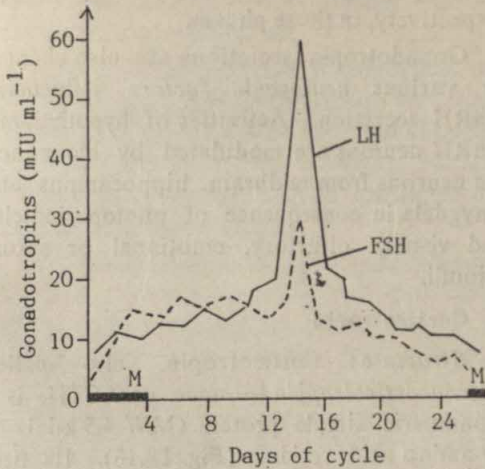


Fig. 18.16. Blood gonadotropin levels in the menstrual cycle. M: menstruation.

women, secretions are pulsatile. During menstruation, FSH secretion shows a small rise, but subsequently declines slowly in the follicular phase while LH secretion starts and continues in a slow rise (Fig. 18.16). Near the middle of the menstrual cycle, there are high, sharp and brief pre-ovulatory rises in the secretions of both FSH and LH. After this midcycle peak, secretions of both fall sharply to low levels and then decline slowly in the later half of the cycle. In both sexes, LH also shows an ultradian rhythm, being secreted in pulses recurring every 90 minutes.

Electrical stimulations of two overlapping zones in the anterior hypothalamus of rats increase the serum concentrations of LH and FSH respectively. Destruction of the median eminence decreases the serum concentrations of both the gonadotropins; but injections of hypothalamic gonadotropin releasing hormone (GnRH) into either the hypophyseal portal vessels or the anterior pituitary itself can still increase the serum gonadotropin concentrations. These indicate the importance of hypothalamic GnRH in regulating FSH and LH secretions. GnRH

activates the phosphatidylinositol- Ca^{2+} -dependent mechanisms in the pituitary gonadotroph cells (page 593). GnRH thereby stimulates the secretion of FSH, LH or both, depending on the feedback effects of the gonadal hormones. It has been suggested that the negative feedback effect of ovarian steroids on short-axon neurons, located near the arcuate nucleus, maintains the basal levels of gonadotropin secretions; the positive feedback effect of ovarian steroids on long-axon supra-chiasmatic neurons may cause the pre-ovulatory upsurge in gonadotropin secretions.

The negative feedback effect of gonadal hormones on the secretion of pituitary gonadotropins is amply demonstrated by the sustained and considerable rise in gonadotropin secretions in both sexes after gonadec-

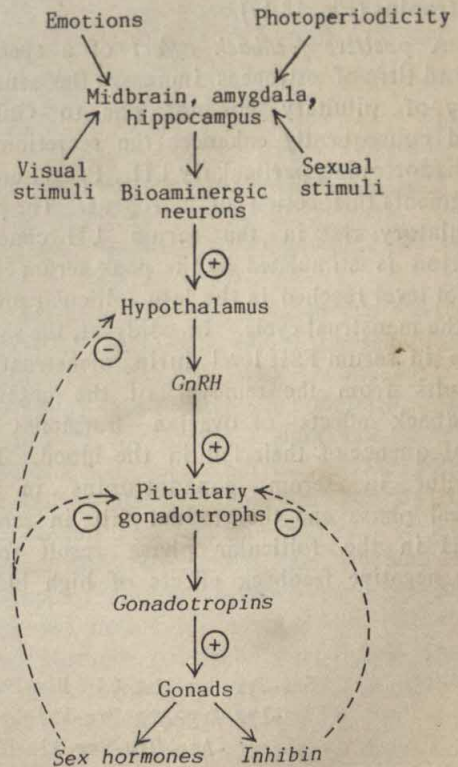


Fig. 18.17. Regulation of gonadotropin secretions.

tomy, the abolition of this rise by the administration of estradiol or testosterone, and the post-menopausal rise in serum gonadotropins. High blood titres of estrogens or testosterone cause a *long-loop feedback inhibition* of both pituitary gonadotrophs and hypothalamic GnRH neurons to reduce gonadotropin secretions. Progesterone causes a similar direct feedback inhibition and also enhances the feedback effect of estrogens. Besides, a peptide called *inhibin* causes a long-loop feedback inhibition of pituitary gonadotrophs to reduce the secretion of FSH, but not of LH. Inhibin is secreted by the testicular Sertoli cells and ovarian follicular cells. Thermal or radiational damages of seminiferous tubules lower inhibin secretion and raise the serum FSH level. In addition, blood FSH titre causes a *short-loop feedback inhibition* of GnRH secretion to reduce the release of gonadotropins (Fig. 18.17).

A *positive feedback effect* of a specific blood titre of estrogens increases the sensitivity of pituitary gonadotrophs to GnRH and consequently enhances the secretion of gonadotropins, particularly LH. Progesterone augments this action of estrogens. The pre-ovulatory rise in the serum LH concentration is stimulated by the peak serum estradiol level reached in the late follicular phase of the menstrual cycle. In contrast, the small rise in serum FSH level during menstruation results from the removal of the negative feedback effects of ovarian hormones in consequence of their fall in the blood. The decline in serum gonadotropins in the luteal phase and the gradual fall in serum FSH in the follicular phase result from the negative feedback effects of high blood

titres of progesterone-estrogen and estrogen, respectively, in those phases.

Gonadotropin secretions are also changed by various *neurogenic factors* influencing GnRH secretion. Activities of hypothalamic GnRH neurons are modulated by bioaminergic neurons from midbrain, hippocampus and amygdala in consequence of photoperiodicity and visual, olfactory, emotional or sexual stimuli.

Corticotropin

Structure: Corticotropin, also called *adrenocorticotrophic hormone* or ACTH, is a monomeric simple protein (MW 4.5 kdal) of 39 amino acid residues (Fig. 18.18). Its first twentyfour N-terminal residues are essential for biological actions and identical among different species. The paired basic amino acids at the 15th-18th positions are required for its complete biological potency. The last fifteen C-terminal residues show considerable species variations and contribute to the immunological specificity. The first thirteen N-terminal residues and the last twentyone C-terminal residues are respectively identical with the amino acid sequences of α -MSH and CLIP.

Synthesis: Cells of anterior and intermediate lobes of the pituitary and some brain cells synthesize a large glycoprotein called *pro-opiomelanocortin* or POMC (≈ 31 kdal) coded by a POMC gene. In different cells, trypsin-like proteases hydrolyze the POMC molecule mostly at different basic amino acid pairs (Lys-Arg, Lys-Lys and Arg-Arg) to give different peptides. In the anterior pituitary, POMC is thus cleaved into one molecule each of ACTH, β -lipotropin (β -LPH) and a

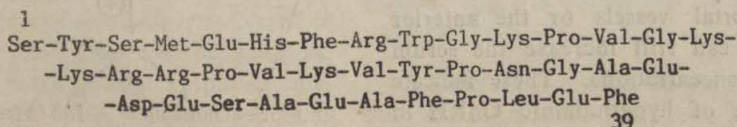


Fig. 18.18. Amino acid sequence of human ACTH.

16-kdal peptide. β -LPH is further cleaved into γ -LPH and *endorphins*.

Actions: The principal physiological role of ACTH is to stimulate the growth and secretion of zona fasciculata of the adrenal cortex. It acts by activating the *adenylate cyclase-cAMP system*; cAMP and its analogues can mimic the effects of ACTH.

(a) Corticotropin binds to specific receptors in the plasma membrane of fasciculata cells to activate adenylate cyclase which increases cAMP in those cells. The activation of adenylate cyclase by ACTH requires the presence of Ca^{2+} . cAMP acts as the second messenger to modify the activities of many enzymes including some protein kinases. This results in an increase in the DNA content, RNA transcription and protein synthesis in those cells. The fasciculata cells consequently proliferate and the *growth of adrenal cortex* is promoted (trophic effect). Hypophysectomy results in the thinning of the z. fasciculata and the atrophy of adrenal cortex; corticotropin administration thickens the z. fasciculata and increases the mass of adrenal cortex.

(b) Corticotropin stimulates the *synthesis and secretion of glucocorticoids* by fasciculata cells. Its maximal steroidogenic effect is attained even when it binds to only a fraction of the corticotropin receptors; so, there are *spare receptors* for this action of ACTH (page 583). The steroidogenic effect is produced in several ways: (i) ACTH increases the transfer of cholesterol from plasma lipoproteins into the fasciculata cells, probably by increasing the lipoprotein receptors on their plasma membrane. (ii) The ACTH-induced rise in cAMP activates a protein kinase which phosphorylates and activates a cholesterol esterase; the latter hydrolyzes cholesteryl esters of intracellular storage pools into free cholesterol. Both these effects enhance the availability of free cholesterol in the fasciculata cells. (iii) Corticotropin promotes the binding of cholesterol to mitochondrial cytochrome P_{450}

required for hydroxylating cholesterol. (iv) It activates the *rate-limiting enzyme* for changing cholesterol to pregnenolone. (v) It also increases the activity of dehydrogenases of the pentose phosphate pathway to make more NADPH available for hydroxylations.

(c) Corticotropin acts directly on adipocytes to activate adenylate cyclase and increase cAMP in them. cAMP in turn mediates in activating the hormone-sensitive lipase which hydrolyzes the stored triacylglycerols into free fatty acids. This mobilizes fatty acids from the adipose tissue into the blood (*adipokinetic effect*). Intravenous ACTH injections elevate the plasma fatty acid level even after adrenalectomy.

β -Lipotropin (β -LPH), a 91-amino acid peptide produced simultaneously with corticotropin, *promotes lipolysis* in adipose tissues to mobilize fatty acids therefrom; but it has little physiological significance. β -LPH mainly serves to produce *endorphins*.

Regulation: Corticotropin secretion has its peak and nadir normally in the morning and at midnight respectively. Plasma ACTH normally averages $<50 \text{ pg ml}^{-1}$, but may reach $>600 \text{ pg}$ in stress.

The hypothalamic *corticotropin releasing hormone* (CRH) stimulates the secretion of POMC, β -lipotropin (β -LPH) and ACTH (see page 592). An "intrinsic biological clock", located in the limbic system and normally reinforced by the diurnal photoperiodicity as well as the rhythmic sleep-activity cycles, maintains a circadian rhythm of CRH release and consequently, the circadian corticotropin rhythm. Stresses such as hypoglycemia and cold increase the release of CRH through particularly the limbic system. Stress consequently enhances the synthesis and release of POMC and ACTH.

The secretion of ACTH is also largely regulated by the feedback effects of gluco-

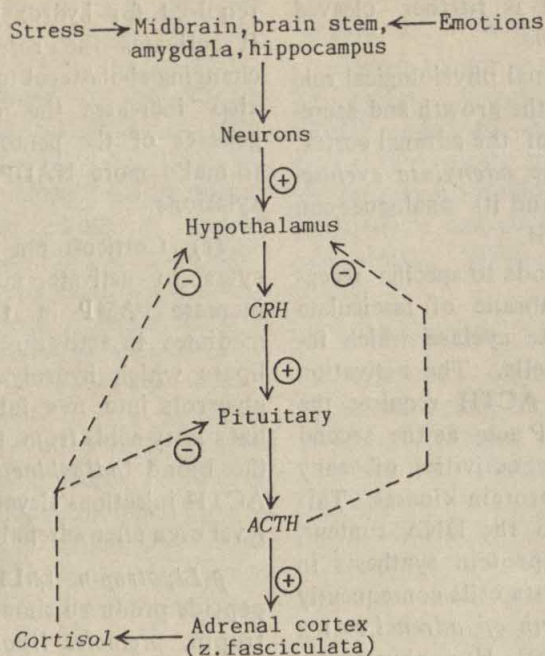


Fig. 18.19. Regulation of ACTH and glucocorticoid secretions.

corticoids like cortisol, and of ACTH itself (Fig. 18.19). Administration of glucocorticoids reduces the firing frequency of hypothalamic neurons, inhibits CRH release and also prevents the rise in POMC and ACTH secretions. Adrenalectomy lowers the blood glucocorticoid level, simultaneously raises plasma CRH and ACTH levels, and manifold enhances the transcription of the POMC gene. Blood glucocorticoids affect both the hypothalamus and the anterior lobe directly. A high blood glucocorticoid level causes *long-loop feedback inhibitions* of the hypothalamic CRH release, the pituitary responsiveness to CRH, and the synthesis as well as secretion of POMC and ACTH. Besides, a rise in blood ACTH level produces a *short-loop feedback inhibition* of hypothalamic CRH release and consequently decreases ACTH secretion.

Cushing's disease: This results from an overproduction of corticotropin due to a

tumor or hyperplasia of β cells of the anterior pituitary. The hypersecretion of corticotropin stimulates excessive secretion of cortical steroids, particularly glucocorticoids; this consequently produces symptoms like hyperglycemia, glucosuria, muscle wasting, atrophy of skin, high urinary NPN, high serum level of free fatty acids, abnormal distribution of body fats, retention of Na^+ and water, and hypertension.

Growth hormone

Structure: Growth hormone or somatotrophin (GH or STH) is a monomeric simple protein secreted by somatotroph α cells of the anterior pituitary. Human GH (MW 21125) has a peptide chain of 191 amino acid residues and carries two intrachain S-S linkages, one forming a small C-terminal loop. It possesses considerable homologies in amino acid sequence and action with prolactin and human chorionic somatomammotropin.

Actions: GH acts by binding to specific membrane receptors on its target cells. But its exact mechanism of action and the second messenger are not yet known. It stimulates the growth of many tissues including muscles, cartilages, mammary glands and bones.

(a) **Protein anabolic action:** GH promotes nitrogen retention and a positive nitrogen balance. It increases the transport of amino acids into muscle cells, lowers the blood amino acid level, depresses protein catabolism to lower the blood NPN, but enhances DNA replication, RNA transcription and translation of proteins in tissues including the liver and muscles to help in tissue growth.

(b) **Actions on carbohydrate metabolism:** GH is antagonistic to insulin in many of its effects. In *gigantism* of pre-adolescent individuals and *acromegaly* of adults, GH hypersecretion produces hyperglycemia, poor sugar tolerance and glucosuria. Hypoglycemia is seen in *Simmond's disease*, caused by GH hyposecretion. Hypophysectomy reduces the severity of pancreatic diabetes while permanent diabetes may result from repeated injections of anterior lobe extracts in a subtotaly pancreatectomized animal. Hypophysectomized-pancreatectomized dogs (*Houssay animals*) have poor sugar tolerance and severe insulin-sensitivity. GH may affect carbohydrate metabolism in several ways: (i) GH produces its *diabetogenic effect*, viz., hyperglycemia and glucosuria, by inhibiting glucose transport into muscle and adipose tissue cells and peripheral utilization of glucose, by increasing hepatic gluconeogenesis and by enhancing mobilization of fatty acids from adipocytes for utilization in place of glucose. (ii) GH reduces insulin-sensitivity and thereby decreases the hypoglycemic effect of insulin (*anti-insulin effect*). (iii) By enhancing gluconeogenesis from amino acids, GH increases the liver glycogen level and reduces its loss during starvation (*glycostatic effect*). It can also increase muscle and cardiac glycogen

level, probably by reducing glycolysis. (iv) GH administration may temporarily raise the release of insulin (*insulinotropic effect*); thus, a transient hypoglycemia may precede the diabetogenic effect.

(c) **Lipolytic action:** GH increases lipolysis in adipocytes by activating the hormone-sensitive *triacylglycerol lipase*. Thus, it mobilizes fatty acids into the blood and raises the plasma free fatty acid and liver lipid levels, leading to increased β -oxidation and ketosis.

(d) **Growth of bones and cartilages:** In *gigantism*, GH hypersecretion prolongs the growth of epiphyseal cartilages to cause overgrowth of long bones and abnormally tall height. In *acromegaly* found in adults, GH hypersecretion abnormally enhances the growth of long bones of lower jaw, arms and legs, which have not completed ossification yet. In *dwarfism*, either GH hyposecretion or tissue refractoriness to GH from childhood produces stunted stature due to premature cessation of growth of the epiphyseal cartilages and consequently of long bones.

(i) The growth effects of GH depend partly on its *calcium anabolic action*. It promotes the retention of Ca^{2+} and PO_4^{3-} in the body to produce a positive calcium balance. This helps in ossification and osteogenesis. (ii) It increases the growth of cartilages and bones by enhancing the incorporation and hydroxylation of proline in the matrix collagen, the incorporation of leucine into glycosaminoglycans of cartilage, the incorporation of SO_4^{2-} into matrix proteoglycans like chondroitin sulfates, the synthesis of DNA and RNA in chondrocytes by incorporating respectively thymidine and uridine in them, and the proliferation (*mitogenic effect*) and growth of chondrocytes. The resulting continuation of the growth of epiphyseal cartilages leads to the elongation of long bones.

The growth effects of GH are mediated by a peptide called *insulin-like growth factor I*

(IGF-I or *somatomedin C*). This monomeric peptide is made of 70 amino acid residues and has close homologies in amino acid sequence with insulin and the peptide IGF-II. GH stimulates cells in the liver and other target tissues to produce and release IGF-I. The latter has local paracrine actions and is also carried by the plasma to other tissues. IGF-I binds strongly to its specific receptor which is a heterodimeric ($\alpha_2\beta_2$) membrane protein; the latter acts as tyrosine kinase when bound to IGF-I (page 581). IGF-I can also bind, though less strongly, to heterodimeric insulin receptors and monomeric IGF-II receptors. IGF-I mimics the actions of GH in incorporating SO_4^{2-} and ^{14}C -leucine into cartilage glycoproteins, ^3H -thymidine and ^3H -uridine into nucleic acids of chondrocytes and fibroblasts, and ^{14}C -proline into collagen. But the GH-induced diabetogenic, lipolytic and ketogenic effects and its stimulation of amino acid uptake by muscle cells appear not to be mediated by IGF-I.

(e) *Mammotropic action*: Because of its sequence homology with prolactin, GH binds to the membrane receptors for prolactin on mammary gland cells, though with a lower affinity, and stimulates some lobulo-alveolar growth in mammary glands.

Regulation: Human GH secretion starts rising within the first hour of deep sleep, shows pulsatile rise every 1-2 hours during deep slumber and may elevate the plasma GH level upto 5 ng ml^{-1} .

Both the transcription of the GH gene in the pituitary somatotroph cells and the release of GH from them are stimulated by the hypothalamic *GH-releasing hormone* or GHRH; *somatostatin* secreted from the hypothalamus inhibits GH secretion (pages 592-593). Many factors like stress, emotions, exercise and sleep rhythm promote GH secretion by stimulating GHRH release through dopaminergic, serotonergic and α -adrenergic nerve impulses from midbrain and limbic system; but

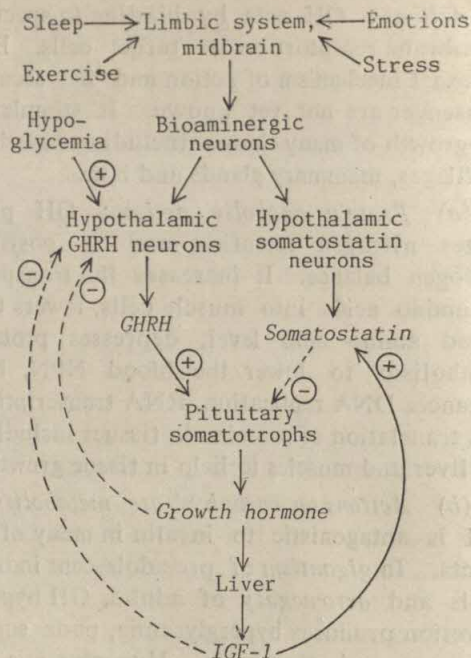


Fig. 18.20. Regulation of growth hormone secretion.

β -adrenergic effects inhibit GHRH release and reduce GH secretion. The glucoreceptors in the ventromedial hypothalamus may be directly stimulated by insulin-induced hypoglycemia and inhibited by glucose-intake hyperglycemia; this influences the GHRH neurons, respectively increasing and decreasing the secretions of GH. GH secretion is also stimulated by elevated plasma levels of arginine or leucine. GH secretion is inhibited by high serum levels of free fatty acids.

IGF-I, released from liver by GH action, may decrease GH secretion by a *long-loop feedback inhibition* of GHRH neurons and a *feedback stimulation* of somatostatin neurons of hypothalamus. GH itself may reduce GH secretion by a *short-loop feedback inhibition* of the hypothalamic GHRH release (Fig. 18.20).

Prolactin

Structure: Prolactin (PRL), also called the *luteotropic hormone* or LTH, is a mono-

meric simple protein ($MW \approx 23$ kdal) made of 199 amino acids and bearing three intrachain S-S linkages. It is secreted by lactotroph α cells of anterior pituitary and has considerable homology of amino acid sequence with GH.

Actions : The major physiological role of prolactin is to stimulate mammary growth and milk secretion.

(a) *Mammotropic and lactogenic actions :* Prolactin binds to specific glycoprotein receptors on the plasma membrane of mammary gland cells. This increases the intracellular concentration of some unknown second messenger which triggers the synthesis of specific mRNA molecules with a consequent enhancement of protein synthesis. This causes the lobulo-alveolar growth of the mammary glands during pregnancy (*mammotropic action*) and the synthesis of milk proteins such as lactalbumin and casein, resulting in milk secretion after parturition (*lactogenic action*). Estrogens, thyroid hormones and glucocorticoids *up-regulate*, i.e., increase the number of prolactin receptors on the mammary cell membrane to augment prolactin action. Prolactin enhances the sensitivity of mammary cells to itself because it can also *up-regulate* its receptors on their plasma membrane. Progesterone reduces the number of prolactin receptors. Consequently, the high blood progesterone level prevents the lactogenic effect of prolactin during pregnancy. With the fall in progesterone titre near parturition, prolactin can initiate lactation.

(b) *Avian cropsac effect :* In pigeons and doves, prolactin stimulates the proliferation and desquamation of the crop epithelium to form *crop milk* which is fed to the young birds.

(c) *Luteotropic action :* In pregnant rats, prolactin enlarges and maintains the corpus luteum in the secretory state in the ovary for a prolonged period. This luteotropic effect is

of little or no significance in women, ungulates and lagomorphs.

(d) *Antigonadotropic action :* On binding to specific glycoprotein receptors on the plasma membranes of human ovarian and testicular cells, prolactin decreases the steroidogenic actions of gonadotropins. It also reduces the release of GnRH from the hypothalamus by decreasing the sensitivity of GnRH neurons to the GnRH-releasing signals. Thus, prolactin exerts antigonadotropic actions in humans. This may explain amenorrhea in lactating women and in women suffering from PRL-secreting tumors.

Regulation : Prolactin secretion normally reaches its peak during sleep. The plasma prolactin concentration averages about 10 ng ml^{-1} in nonpregnant and nonlactating women. It rises progressively from the first trimester of pregnancy and reaches a mean of about 200 ng ml^{-1} at parturition.

Hypothalamus regulates prolactin secretion mainly by releasing some *prolactin release-inhibiting hormones* (PRIH). Transection of the pituitary stalk or transplantation of the pituitary elsewhere from the sella turcica increases the blood prolactin level. *Dopamine*, released from hypothalamic dopaminergic neurons, may act as a PRIH by inhibiting lactotroph cells (page 593). It reduces PRL synthesis by inhibiting the transcription of PRL gene in lactotrophs and decreases the release of PRL from them. Dopaminergic agents and dopamine antagonists respectively reduce and enhance PRL secretion. *GnRH-associated peptide* (GAP) may act as another PRIH.

Prolactin releasing hormones (PRH), e.g., *TRH*, released from the hypothalamus due to serotonergic nerve impulses reaching there, may stimulate prolactin release.

Neuroendocrine reflexes, stimulated by suckling, coitus or nipple manipulation, inhibit

thereby reduces the mobilization of Ca^{2+} and Pi from bones into the blood. CT action decreases the activities of osteoclasts, the ruffling and microvilli on their membrane, activities of lysosomal hydrolases, alkaline phosphatase and pyrophosphatase in bones, collagen metabolism and urinary hydroxyproline. It also stimulates phosphate influx into bone cells. Although calcitonin inhibits the stimulating action of PTH on osteoclasts, it seems not to influence osteoblasts.

The calcium-retaining action of CT may be significant in pregnant and lactating women, growing children and egg-laying birds.

(b) *Action on kidneys*: By acting on the thick ascending limb of Henle's loop and the distal tubule of the kidney, calcitonin decreases tubular reabsorption of Ca^{2+} and Pi from the urine and thereby enhances their renal clearances. This may also contribute to its hypocalcemic effect.

Regulation :

Calcitonin is secreted continuously under normal conditions. Rises in the serum Ca^{2+} level stimulate linear rises in CT secretion while fall in serum Ca^{2+} decreases its secretion. Infusion of Ca^{2+} into rats causes degranulation, hypertrophy and hyperplasia of C cells, enlargement of their rough ER and Golgi cisternae, and rise in serum CT level. Low serum Ca^{2+} leaves C cells unchanged and the serum CT declines.

Glucagon and β -effects of catecholamines stimulate CT secretion by activating the adenylate cyclase-cAMP system in C cells. Gastrin and cholecystokinin stimulate CT secretion while somatostatin inhibits it.

18.11 PARATHYROID HORMONE

Parathyroid hormone (PTH) or parathormone is secreted by the chief cells of parathyroids.

Structure and synthesis :

PTH is a monomeric polypeptide (MW 9.5 kdal) made of 84 amino acids and free from any prosthetic group. Its entire amino acid sequence is not necessary for its full biological activity. Its N-terminal stretch, consisting of the first 34 amino acid residues, possesses the receptor-binding ability and exerts the full hypercalcemic and phosphaturic effects.

Polysomes, adhering on the rough ER membrane, translate *preproPTH* consisting of 115 amino acids. Before its translation is completed, its N-terminal end protrudes into the lumen of the rough ER and a *signal peptidase* of the rough ER membrane hydrolytically cleaves off the 25-amino-acid signal peptide segment, rich in hydrophobic amino acids, from the N-terminal end of *preproPTH*. This changes the latter to *proPTH* made of 90 amino acids. After its transfer into the rough ER lumen, *proPTH* moves to the Golgi cisternae. There, a trypsin-like protease called *clipase B* hydrolytically removes its N-terminal hexapeptide segment rich in basic amino acids, changing *proPTH* to PTH. The latter is packaged and stored in secretory vesicles pinched off from Golgi cisternae. Either cAMP or a low Ca^{2+} concentration stimulates the margination of secretory vesicles in the chief cell and the fusion of their membrane with the plasma membrane to release PTH outside the cell. But a high Ca^{2+} concentration stimulates the degradation of the stored PTH in secretory vesicles, instead of its release. On the contrary, the synthesis of *preproPTH* and *proPTH* is a *constitutive* process, continuing at an unchanged rate inspite of changes in the serum Ca^{2+} concentration.

Actions :

PTH increases the serum Ca^{2+} level by acting on bones, kidneys and intestine. It binds to a species of PTH receptors (MW 70 kdal) on the plasma membrane of bone

cells and renal tubule cells, and thereby activates the membrane *adenylate cyclase* to generate cAMP in the cells. cAMP acts as the second messenger to activate specific protein kinases which phosphorylate and thereby modulate the activities of specific proteins in the bone and kidney cells. cAMP also increases the Ca^{2+} concentration in these cells; Ca^{2+} in turn may act as a messenger to modulate the activities of some intracellular proteins.

(a) *Action on bones*: PTH binds to specific membrane receptor proteins on osteoclasts, osteocytes and osteoblasts to increase the intracellular cAMP which then affects their activities in four major ways. (i) PTH stimulates osteoclasts to increase the resorption of bones by them (*osteoclastic osteolysis*) and consequently enhances the mobilization of Ca^{2+} and Pi from bones into the blood. Its action on osteoclasts increases their number, activity and microvilli, and activities of lysosomal hydrolases and carbonic anhydrase. (ii) PTH also stimulates the resorption of bones by osteocytes (*osteocytic osteolysis*) with the consequent release of bone minerals into the blood and enlargement of bone lacunae. (iii) It may stimulate the differentiation and maturation of precursor cells into osteoclasts. (iv) At higher levels of physiological concentrations, PTH inhibits alkaline phosphatase activity and collagen synthesis in osteoblasts and consequently decreases the Ca^{2+} -retaining capacity of bones. But at low concentrations, PTH stimulates the sulfation of cartilages and increases the number and alkaline phosphatase activity of osteoblasts, helping in forming and remodelling bones.

The initial phase of the PTH-mediated mobilization of Ca^{2+} seems to be independent of RNA and protein synthesis, but its later phase depends on such synthesis and is inhibited by actinomycin D.

(b) *Action on kidneys*: PTH binds to

the PTH receptors on the basal and lateral plasma membrane of renal cortical cells of both proximal and distal tubules. This activates the adenylate cyclase in their basolateral membrane, leading to the generation of cAMP in the basal cytoplasm. cAMP is thence transported to the apical or luminal part of the cell where it allosterically activates cAMP-dependent protein kinases. These phosphorylate and thereby modulate specific proteins of the apical membrane to affect the transport of several minerals across that membrane. In this way, (i) PTH decreases the transmembrane transport and reabsorption of filtered Pi in both proximal and distal cortical tubules and increases the urinary elimination of Pi (*phosphaturic effect*). The consequent fall in serum Pi level leads to the mobilization of Pi, accompanied by Ca^{2+} , from bones and thus to hypercalcemia. (ii) PTH increases the transmembrane transport and reabsorption of filtered Ca^{2+} in the distal tubules and decreases the urinary elimination of Ca^{2+} . However, PTH-induced hypercalcemia may enhance the amount of filtered Ca^{2+} to raise the renal excretion of the latter. The overall effect of PTH on urinary Ca^{2+} elimination depends on the balance between these counteracting effects and ordinarily decreases the renal clearance of Ca^{2+} . (iii) PTH inhibits the transmembrane transport of K^+ and HCO_3^- to decrease their reabsorption by renal tubule cells. (iv) PTH stimulates the activity of 25-HCC 1 α -hydroxylase in mitochondria of the proximal convoluted tubule cells. This enzyme converts 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol (page 170). Thus, PTH enhances the renal formation of 1,25[OH] $_2$ -D $_3$ (calcitriol) which in turn increases the intestinal and renal absorptions of Ca^{2+} and Pi and also affects the deposition as well as mobilization of bone Ca^{2+} (page 172).

(c) *Action on intestinal mucosa*: PTH does not act directly on the intestinal mucosa

cells which do not seem to possess PTH receptors. But it indirectly enhances the intestinal absorption of Ca^{2+} and Pi, because it stimulates the renal formation of calcitriol ($1,25[\text{OH}]_2\text{-D}_3$).

Hypoparathyroidism :

Deficiency of PTH may result from an autoimmune destruction of parathyroids (*primary hypoparathyroidism*), an accidental removal of parathyroids during thyroid surgery (*secondary hypoparathyroidism*) or a failure of parathyroid function due to unknown causes (*ideopathic hypoparathyroidism*). Hypoparathyroidism lowers the serum Ca^{2+} and raises the plasma Pi. The low serum Ca^{2+} causes neuromuscular hyperexcitability, resulting in paraesthesia and muscular spasms in larynx, extremities and face (*parathyroid tetany*).

Pseudohypoparathyroidism :

In this congenital disorder, there is no deficiency of PTH secretion; but bones and kidneys are resistant to PTH action due frequently to an inherited deficiency of the GTP-dependent regulatory protein (G_s or N_s) participating in the activation of the membrane adenylate cyclase of target cells by PTH (page 578). Symptoms include low serum Ca^{2+} , high serum Pi, neuromuscular hyperexcitability, short metacarpals and metatarsals, short height, mental retardation and rounded face; serum PTH rises because hypocalcemia stimulates parathyroids.

Hyperparathyroidism :

Oversecretion of PTH may result from a parathyroid hyperplasia or tumor (*primary hyperparathyroidism*) or from a chronic renal failure with poor formation of $1,25[\text{OH}]_2\text{-D}_3$ (*secondary hyperparathyroidism*). PTH oversecretion produces *osteitis fibrosa cystica*, characterized by high urinary and serum Ca^{2+} , low serum Pi, decalcification and softening of bones, metastatic calcification of

soft tissues, urinary calculi and gastrointestinal hypotonia.

Regulation :

PTH is synthesized and secreted continually. Its secretion is mainly under the negative feedback control of serum Ca^{2+} concentration—a rise in serum Ca^{2+} inhibits and a fall in serum Ca^{2+} stimulates PTH secretion. Serum Mg^{2+} has similar but weaker effects on PTH secretion. PTH secretion rises on reducing the serum Ca^{2+} by injections of EDTA, oxalate or fluoride and bears a positive linear correlation with the intracellular cAMP level in parathyroids. cAMP seems to be a mediator of the action of serum Ca^{2+} on PTH secretion. Rise in serum Ca^{2+} leads to a rise in intracellular Ca^{2+} which may lower the intracellular cAMP by inhibiting adenylate cyclase or by activating cAMP phosphodiesterase; the fall in cAMP level may reduce PTH secretion. A lowering of serum Ca^{2+} may produce reverse effects. PGE_1 , dopamine and β -effects of catecholamines increase PTH secretion by enhancing cAMP in the parathyroid cell.

In addition, Ca^{2+} decreases PTH secretion by activating proteolytic enzymes which degrade PTH molecules stored in the secretory vesicles of parathyroid cells. Poor intake of Ca^{2+} lowers the Ca^{2+} concentration in those cells and consequently reduces the degradation of PTH, making more PTH available for secretion.

18.12 ADRENAL MEDULLARY HORMONES

Chromaffin cells of adrenal medulla secrete two catecholamine hormones, *adrenaline* (epinephrine) and *noradrenaline* (norepinephrine). In most adult mammals, adrenaline is the principal medullary hormone. Noradrenaline predominates in mammalian fetus, whales, reptiles, dogfishes and chicks. It is also released at adrenergic nerve endings.

Synthesis and catabolism :

Catecholamines are synthesized from tyrosine in chromaffin cells (page 377 and Fig. 12.22). The synthesis is carried out partly in the cytoplasm and partly in the secretory vesicles called chromaffin granules. These granules store catecholamines along with proteins called *chromogranins*, Mg^{2+} -ATP and Ca^{2+} . Acetylcholine, released in the adrenal medulla from cholinergic sympathetic nerve terminals, depolarizes the membrane of chromaffin cells and enhances Ca^{2+} influx into them. Ca^{2+} stimulates the release of catecholamines by the fusion of the membranes of chromaffin granules with the plasma membrane. Catecholamines are carried by serum albumin in the plasma and have an average half-life of about 20 seconds. They are degraded in the blood, liver, heart, muscles and other tissues into vanilmandelate and other products which are excreted in the urine (pages 377-378).

Actions :

Stress stimulates the secretion of adrenaline from adrenal medulla and the release of noradrenaline from adrenergic sympathetic neurons. They act mostly in close coordination to help in combating stress.

Adrenergic receptors or *adrenoreceptors* are classified on the basis of the pharmacological agents acting as their antagonists.

(i) α -*Adrenergic receptors* are blocked competitively by phentolamine and ergot alkaloids like dihydroergotamine, and non-competitively by phenoxybenzamine; these agents are therefore called α -blockers. Of the α -adrenoreceptors, α_1 and α_2 receptors are blocked selectively by prazosin and yohimbine respectively. α_1 -Adrenoreceptors are very sensitive to adrenaline, equally or less sensitive to noradrenaline and far less sensitive to isoproterenol. They predominate on the postsynaptic membranes of smooth muscles and generally excite the contraction of smooth

muscles when bound to the catecholamines. α_2 -Adrenoreceptors are more sensitive to adrenaline in some tissues and to noradrenaline in others, but are almost insensitive to isoproterenol. They occur on the presynaptic neuronal membrane where they participate in the feedback inhibitory effect of noradrenaline on the presynaptic neuron. α_2 -Adrenoreceptors also occur on the postsynaptic membranes of adipocytes and some gland cells and mediate inhibitory effects of catecholamines on adipose tissue lipolysis and some glandular secretions.

(ii) β -*Adrenergic receptors* are blocked by β -blockers such as pronethalol, dichloroisoproterenol and propranolol. β_1 and β_2 adrenoreceptors are blocked selectively by practolol and butoxamine respectively. β_1 -Adrenoreceptors are almost equally sensitive to adrenaline and noradrenaline, but much more sensitive to isoproterenol. They occur in high density in the heart and are generally excitatory to the latter. β_2 -Adrenoreceptors are slightly more sensitive to isoproterenol than to adrenaline, but much less sensitive to noradrenaline. They occur mainly on smooth muscle cells and gland cells. When acted upon by catecholamines, they generally relax the smooth muscles but excite the secretions of many gland cells like the pancreatic islet cells, renal juxtaglomerular cells and thyroid cells.

At physiological concentrations, noradrenaline binds to and activates mainly the α receptors; adrenaline binds to both α and β receptors and the tissue response to it depends on their relative concentrations on the target cell membrane and their relative affinities for adrenaline. Evidently, a tissue lacking in α receptors will be little affected by noradrenaline.

On binding to β_1 and β_2 receptors, catecholamines activate adenylate cyclase in the target cell membrane (page 578). Adenylate cyclase activity increases the intracellular

concentration of cAMP. The latter allosterically activates specific protein kinases which phosphorylate specific proteins or enzymes, activating some of them and inactivating others. This leads to the biological responses of the tissue. Chronic rises in blood catecholamine concentrations down-regulate the number of β receptors on target cells, and also reduce the activity of the remaining β receptors by uncoupling them from adenylate cyclase. Thyroid hormones increase the β effects of catecholamines by up-regulating the number of β -adrenergic receptors and facilitating their coupling with adenylate cyclase.

On binding to α_2 receptors, catecholamines inhibit adenylate cyclase to reduce the intracellular cAMP concentration; this leads to the tissue responses in α_2 effects. But α_1 effects are not mediated by the adenylate cyclase-cAMP system. On binding with α_1 receptors, catecholamines affect the formation of inositol 1,4,5-triphosphate and diacylglycerol, and/or the intracellular concentration of Ca^{2+} ; these may act as second messengers to produce tissue responses during α_1 effects.

(a) *Vascular actions*: Adrenaline is principally a *vasodilator*, relaxing the smooth muscles on many vessels including those of skeletal muscles (β_2 effect). Although it may also contract vascular smooth muscles in the skin, kidneys, abdominal viscera and gastrointestinal mucosa to cause their *vasoconstriction* (α effect), adrenaline decreases the overall peripheral resistance because of its predominant vasodilator effect and may consequently lower the diastolic pressure. Noradrenaline is predominantly a *vasoconstrictor* (α_1 effect) and raises the peripheral resistance to increase thereby both systolic and diastolic blood pressures.

(b) *Cardiac actions*: Adrenaline enhances the rhythmicity and contractility of the heart (β_1 effect) and consequently increases the rate

(*chronotropic effect*) and force (*inotropic effect*) of heart beats to raise the cardiac output and the systolic blood pressure. Noradrenaline has almost no direct action on the heart muscle and has little effect on cardiac output.

(c) *Smooth muscle actions*: Adrenaline relaxes the smooth muscles of gastrointestinal tract through α_2 and β_2 effects, and those of urinary bladder, bronchioles, ciliary muscles and nonpregnant uterus of cat or rat through β_2 effects. But it contracts the smooth muscles of gastrointestinal and bladder sphincters, dilator pupillae, spleen, pilomotor muscles, pregnant uterus of rat or cat, and both pregnant and nonpregnant uteri of women (α_1 effects). Noradrenaline does not relax smooth muscles on blood vessels and bronchi, because β_2 receptors involved in their relaxation are very little affected by moderate doses of noradrenaline. Noradrenaline has relatively weak inhibitory effects on other smooth muscles also, because both α_1 and β_2 receptors on them are much more sensitive to adrenaline than to noradrenaline.

(d) *Glycogenolytic action*: Adrenaline increases blood glucose by enhancing hepatic glycogenolysis through its binding to β_2 and α_1 receptors on hepatocyte membrane. The β_2 effect of adrenaline produces a cAMP-mediated activation of liver glycogen phosphorylase to enhance glycogenolysis. In the rat liver, however, the α_1 effect of adrenaline increases the intracellular concentration of Ca^{2+} which also leads to the activation of glycogen phosphorylase and a consequent rise in glycogenolysis (page 316). Noradrenaline has very little effect on blood glucose.

(e) *Lipolytic action*: Both adrenaline and noradrenaline predominantly stimulate adipose tissue lipolysis and thereby increase nonesterified fatty acids in the blood (β_1 effect). The catecholamine-induced rise in cAMP leads to the activation of triacylglycerol lipase in adipocytes to enhance lipolysis (page 451).

In some cases, catecholamines may inhibit lipolysis by decreasing the intracellular cAMP concentration (α_2 effect).

(f) *Gluconeogenic action*: Adrenaline increases hepatic gluconeogenesis (β_2 effect). Adrenaline-stimulated rise in cAMP induces the synthesis of pyruvate carboxylase, fructose 1,6-bisphosphatase and PEP carboxykinase, all key enzymes for gluconeogenesis (page 346). Moreover, the rise in blood free fatty acid level produced by the β_1 -mediated lipolytic action of adrenaline, can also activate hepatic gluconeogenesis.

(g) *Glycolytic action*: Adrenaline increases blood lactate by promoting muscle glycolysis, but noradrenaline has very little effect on blood lactate. In skeletal and cardiac muscles, adrenaline increases glycolysis mainly through a cAMP-mediated activation of glycogen phosphorylase (β_2 effect). But in smooth muscles, it increases glycolysis through a Ca^{2+} -mediated activation of the phosphorylase (α_1 effect). (See page 324).

(h) *Calorigenic action*: Adrenaline increases O_2 consumption, BMR and body temperature, by increasing glycolysis and fatty acid oxidation (β effects) and by reducing heat loss through cutaneous vasoconstriction (α effect).

Pheochromocytoma:

This is a chromaffin cell tumor in the adrenal medulla or elsewhere. Catecholamine secretions, blood and urinary levels of catecholamines and vanilmandelates, blood glucose, blood non-esterified fatty acids, heart rate, BMR and blood pressure rise abnormally. Glucosuria and ventricular fibrillations also occur.

Regulation:

Catecholamine secretion increases markedly under stress conditions like hypoglycemia, hemorrhage, trauma and cold. Stress causes ACTH and glucocorticoid secretions and sends cholinergic sympathetic impulses to the adrenal medulla. Acetylcholine, released at

nerve endings, as well as ACTH increases the synthesis of tyrosine hydroxylase in the chromaffin cells to enhance catecholamine synthesis. Glucocorticoids increase catecholamine synthesis by enhancing the activities of most of the enzymes of that pathway, particularly phenylethanolamine N-methyltransferase. Acetylcholine from sympathetic nerve terminals increases Ca^{2+} influx into the chromaffin cells. This enhances the release of catecholamines from chromaffin granules.

18.13 ADRENAL CORTEX HORMONES

Structure and classification:

Adrenal cortex secretes three groups of steroids: (i) *Glucocorticoids* are C_{21} steroids with either $-\text{OH}$ or $=\text{O}$ at C^{11} . They influence the carbohydrate metabolism and many other activities. *Cortisol* is the major glucocorticoid in man, goat, sheep, dog and guinea pig, *corticosterone* in rat, rabbit, mouse, duck, chick, reptiles and amphibians, and both cortisol and corticosterone in cattle, pig and cat. Presence of a $\text{C}^{17}\text{-OH}$ group increases glucocorticoid activity; e.g., cortisol with a $\text{C}^{17}\text{-OH}$ is much more potent as a glucocorticoid in humans than corticosterone without the $\text{C}^{17}\text{-OH}$. (ii) *Mineralocorticoids* are C_{21} steroids which influence mainly the metabolisms of Na^+ and K^+ . *Aldosterone*, the principal mineralocorticoid in humans, most other mammals, birds, reptiles and amphibians, bears a $\text{C}^{11}\text{-OH}$ group and a $\text{C}^{18}\text{-aldehyde}$ group. Other mineralocorticoids such as *11-deoxycorticosterone* (DOC) and *11-deoxycortisol* carry neither $-\text{OH}$ nor $=\text{O}$ at C^{11} . (iii) *Sex corticoids* are C_{18} and C_{21} steroids which influence accessory sex characters. They include *dehydroepiandrosterone* (DHEA), *androstenedione* and *estradiol* (all C_{18}), and *progesterone* (C_{21}).

Synthesis:

Corticosteroids are synthesized from cholesterol in the adrenal cortex (Fig. 18.22).

In all the three zones of adrenal cortex, cholesterol is first changed to pregnenolone (C_{21}) by a common pathway. For this, free cholesterol is released in the cytoplasm from cholesteryl esters of cytoplasmic lipid droplets and transferred into mitochondria. A 20,22-desmolase or cytochrome P_{450} sidechain cleavage enzyme ($P-450_{scc}$) of the inner mitochondrial membrane hydroxylates cholesterol at C^{22} and C^{20} , and then cleaves its sidechain to give isocaproic aldehyde

and pregnenolone; this is the *rate-limiting step* for corticosteroid biosynthesis. $P-450_{scc}$ uses NADPH and molecular O_2 and includes an FAD-containing flavoprotein, an Fe_2S_2 -protein (adrenodoxin) and cytochrome P_{450} as its components (page 288). The subsequent pathway differs in the three zones.

(a) *Glucocorticoid synthesis*: Glucocorticoids are synthesized in zona fasciculata cells. Pregnenolone is transferred to the smooth endoplasmic reticulum. There, it is mostly

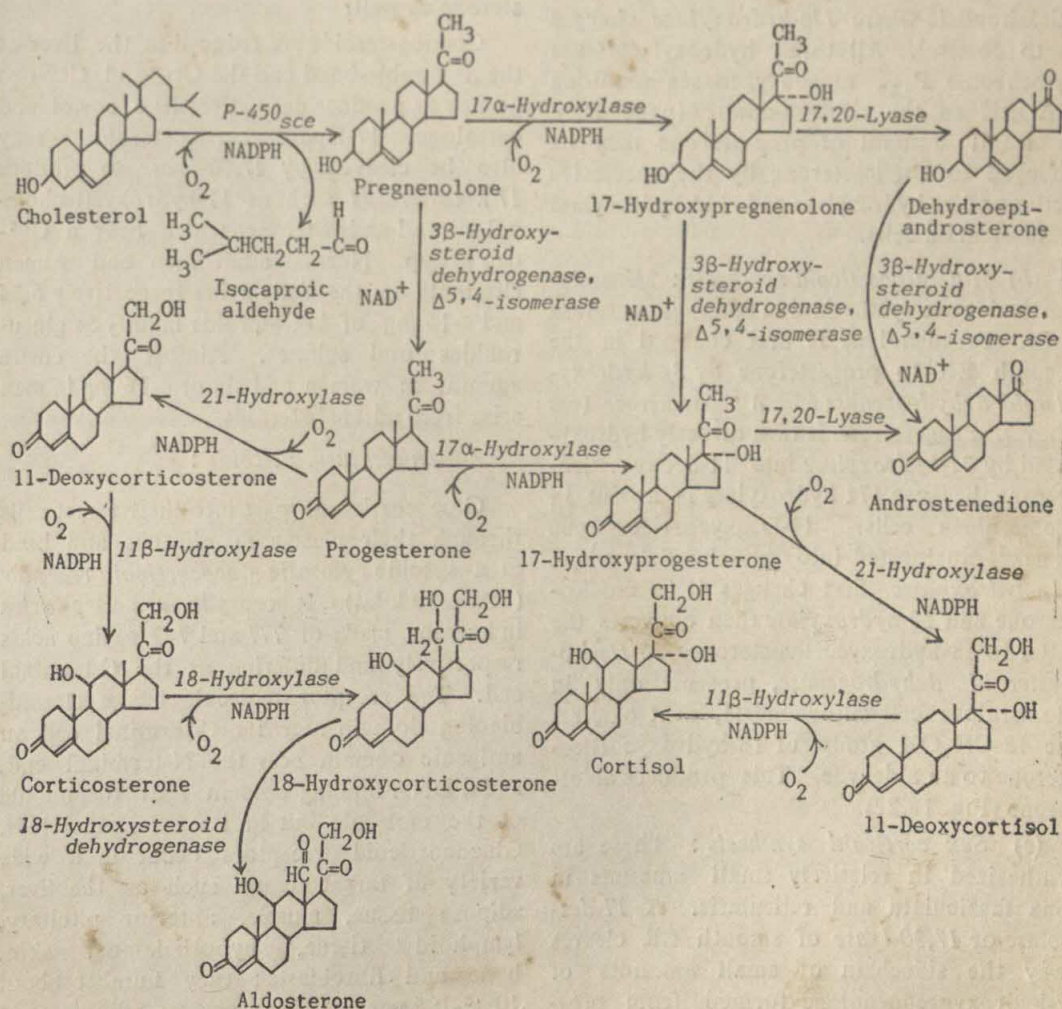


Fig. 18.22. Biosynthesis of adrenal corticosteroids.

hydroxylated by *17 α -hydroxylase* to form 17-hydroxypregnenolone. The latter is then changed to 17-hydroxyprogesterone by the successive actions of the NAD⁺-dependent *3 β -hydroxysteroid dehydrogenase* and *$\Delta^{5,4}$ -isomerase*. Alternatively, some pregnenolone may be changed first by the dehydrogenase and the isomerase to progesterone which is then hydroxylated by *17 α -hydroxylase* to 17-hydroxyprogesterone. 17-Hydroxyprogesterone is next changed by *21-hydroxylase* of ER into 11-deoxycortisol which is translocated into mitochondria where *11 β -hydroxylase* changes it to cortisol. All three hydroxylases are cytochrome P₄₅₀ monooxygenases requiring NADPH as the electron-donor (page 288). A small amount of progesterone may be changed to corticosterone by the successive actions of *21-hydroxylase* and *11 β -hydroxylase* in fasciculata cells.

(b) *Mineralocorticoid synthesis*: Mineralocorticoids are synthesized in zona glomerulosa cells. Pregnenolone is first changed in the smooth ER to progesterone by *3 β -hydroxysteroid dehydrogenase* and *$\Delta^{5,4}$ -isomerase* (see above). Progesterone is then directly hydroxylated by *21-hydroxylase* into 11-deoxycorticosterone, because *17 α -hydroxylase* is absent in glomerulosa cells. 11-Deoxycorticosterone is next translocated into mitochondria where *11 β -hydroxylase* first changes it to corticosterone and *18-hydroxylase* then converts the latter to 18-hydroxycorticosterone. *18-Hydroxysteroid dehydrogenase*, present only in glomerulosa cell mitochondria, next oxidizes the 18-CH₂OH group of 18-hydroxycorticosterone to an aldehyde. This produces aldosterone (Fig. 18.22).

(c) *Sex corticoid synthesis*: These are synthesized in relatively small amounts in zona fasciculata and reticularis. A *17-desmolase* or *17,20-lyase* of smooth ER cleaves away the sidechain of small amounts of 17-hydroxypregnenolone formed from pregnenolone in the glucocorticoid pathway; this

produces dehydroepiandrosterone which is oxidized and isomerized to androstenedione by the successive actions of *3 β -hydroxysteroid dehydrogenase* and *$\Delta^{5,4}$ -isomerase* (Fig. 18.22).

Transport and catabolism:

Each dl of plasma carries about 0.01 μ g of aldosterone weakly bound to serum albumin, about 0.5-0.8 μ g of free cortisol and 10-15 μ g of cortisol bound to an α_1 -globulin, *transcortin* (52 kdal). The latter carries deoxycorticosterone and corticosterone as well.

Corticosteroids are reduced in the liver at the Δ^4 double-bond and the C³- and C²⁰-oxo groups to produce derivatives like cortol and cortolone. 17-Hydroxycorticosteroids may also be cleaved by *17,20-lyase* to produce *17-ketosteroids* such as 11-hydroxyetiocholanolone and androsterone, which bear a C¹⁷-oxo group. Normal adult men and women eliminate in the daily urine respectively 6-24 and 4-16 mg of ketosteroids mainly as glucuronides and sulfates. Almost the entire amount in women and about 4-16 mg in men arise from adrenal steroids.

Action of glucocorticoids:

Glucocorticoids pass into their target cells through their plasma membranes and bind to a specific cytosolic *glucocorticoid receptor* (MW $\approx$ 90 kdal). It occurs in α and β forms in humans, made of 777 and 742 amino acids respectively and differing at the C-terminal end. The receptor molecule has a steroid-binding domain near its C-terminal end, an antigenic domain near the N-terminal end, and a DNA-binding domain near the middle of the molecule but in its C-terminal half. Glucocorticoid receptors occur in a wide variety of target tissues such as the liver, adipose tissue, muscle, anterior pituitary, lymphoid tissue, hypothalamus, skin, bone and fibroblasts; they number about 10^{3.5}-10⁵ per cell. They possess affinities not only for cortisol and corticosterone, but

also for aldosterone and other mineralocorticoids; but these mineralocorticoids normally have no significant glucocorticoid action because of their much lower concentration in the plasma and much higher EC_{50} (page 582) than cortisol. Sex corticoids are either antagonists or inactive agents for glucocorticoid receptors. Because of its much higher plasma concentration, cortisol exerts some mineralocorticoid actions even normally, although it binds to mineralocorticoid receptors with a much weaker affinity than aldosterone.

Binding of a glucocorticoid to the glucocorticoid receptor changes the conformation of the latter, enabling its DNA-binding site to bind to the *hormone-responsive element* (HRE) of specific nuclear genes (page 577). This modulates the transcription rates of those genes, leading not only to the increased synthesis of many specific proteins (e.g., key gluconeogenic enzymes and tyrosine transaminase), but also to the decreased synthesis of some proteins like corticotropin. Besides, glucocorticoids also modulate the post-transcriptional processing of specific RNA transcripts, the degradation and half-life of some specific mRNAs and even the post-translational processing of specific proteins. All these lead to the biological effects of glucocorticoids.

(a) *Carbohydrate metabolism*: Glucocorticoids increase the blood sugar level in several ways. (i) They increase gluconeogenesis in the liver by inducing the synthesis of key gluconeogenic enzymes such as PEP carboxykinase, pyruvate carboxylase, fructose 1,6-diphosphatase and glucose 6-phosphatase through the enhanced transcription of their genes, and also by reducing the rate of degradation of the PEPCK mRNA so as to prolong its half-life. (ii) They increase gluconeogenesis also by making more gluconeogenic metabolites available. This they do by decreasing amino acid incorporation into proteins,

increasing protein catabolism in extrahepatic tissues like muscles, adipocytes, lymphoid tissues, skin and bones, and also by inducing the gene transcription and synthesis of some rate-limiting enzymes for amino acid catabolism such as tryptophan 2,3-dioxygenase, tyrosine transaminase and alanine transaminase. (iii) Glucocorticoids also decrease glucose uptake and utilization in muscles, adipocytes and lymphoid cells by inhibiting the membrane transport of glucose into those cells. All these effects can produce hyperglycemia and glucosuria in cases of cortisol hypersecretion (Cushing's syndrome), or on prolonged administration of cortisol in excessive doses to insulin-deficient animals. The balance between the hyperglycemic effect of cortisol and the hypoglycemic effect of insulin maintains the normal blood sugar. Cortisol deficiency in Addison's disease produces hypoglycemia and enhances insulin-sensitivity.

Glucocorticoids increase the hepatic glycogen store by (i) enhancing gluconeogenesis from amino acids, (ii) inducing the gene transcription and synthesis of glycogen synthase, the rate-limiting enzyme for glycogenesis, and (iii) activating the protein phosphatase which dephosphorylates and activates glycogen synthase.

Glucocorticoids help to maintain blood glucose and liver glycogen, particularly during fasting. Cortisol-deficient individuals show rapid decline in liver glycogen and even a fatal hypoglycemia during fasting.

(b) *Protein metabolism*: In extrahepatic tissues like muscles, adipocytes and lymphoid tissues, cortisol increases protein catabolism by (i) decreasing amino acid incorporation into proteins, and (ii) inducing gene transcription and synthesis of tyrosine transaminase, tryptophan 2,3-dioxygenase and alanine transaminase which catalyze the rate-limiting steps of amino acid catabolism. On the contrary, cortisol increases protein ana-

bolism in the liver by enhancing (i) amino acid transport into hepatocytes, (ii) gene transcription of mRNAs for specific proteins, (iii) incorporation of amino acids into ribosomal proteins, and (iv) synthesis of many proteins including plasma proteins. But (v) cortisol simultaneously increases *urea synthesis* in the liver by inducing the synthesis of argininosuccinate synthase, argininosuccinate lyase and arginase. The over-all effect of cortisol is to produce a *negative N balance*.

(c) *Lipid metabolism*: (i) Glucocorticoids enhance the lipolysis of fat into fatty acids by facilitating the activation of triacylglycerol lipase in adipocytes by adrenaline, glucagon and growth hormone. (ii) By decreasing glucose uptake by adipocytes, glucocorticoids reduce the production of glycerol α -phosphate, without which fatty acids cannot be esterified for retention in those cells. Due to these effects, glucocorticoids increase the *mobilization of fatty acids* from adipocytes and elevate the plasma nonesterified fatty acid level. They may also affect the *distribution of body fat*; fat accumulates abnormally on the face, neck, back and abdomen in Cushing's syndrome due to cortisol hypersecretion.

(d) *Immunosuppression*: High doses of glucocorticoids decrease gene transcription, protein synthesis and antigen-induced proliferation of lymphocytes in thymus, lymph glands and spleen, and shift lymphocytes from circulation to lymphoid tissues. The lymphoid tissue mass, blood lymphocyte count, T cell-mediated cellular immunity and graft rejection are also reduced. These suppress the immune response.

(e) *Anti-inflammatory action*: Glucocorticoids in large doses suppress inflammation because they (i) block the release of lysosomal hydrolases by increasing the resistance of lysosomal membranes to lysis, (ii) inhibit the formation of kinins, (iii) reduce vascular permeability to prevent extravasation of fluids into tissues, (iv) decrease the numbers

of circulating monocytes, eosinophils and lymphocytes, (v) decrease the aggregation of granulocytes in the affected tissue in spite of increasing their release into blood from bone marrow, (vi) inhibit the release of interleukin I from granulocytes, (vii) decrease the production of prostaglandins, prostacyclin, leukotrienes and thromboxanes by inhibiting phospholipase A_2 required for their synthesis, (viii) reduce fibroblast proliferation and collagen synthesis, and (ix) prevent the release of histamine from mast cell granules.

Actions of mineralocorticoids:

Entering through the plasma membranes of their target cells, mineralocorticoids bind to specific *mineralocorticoid receptors* in the cytosol and nucleoplasm. Both high-affinity and low-affinity mineralocorticoid receptors occur in only a few epithelia such as those of the renal distal tubules and collecting ducts, salivary gland ducts, sweat glands and gastrointestinal mucosa. The steroid-receptor complex goes to bind to the hormone-responsive element (HRE) of specific nuclear genes and thereby increases the transcription rates of those genes.

(a) Aldosterone and far less, deoxycorticosterone decrease the elimination of Na^+ in urine, sweat, saliva and gastric juice by promoting the *reabsorption of Na^+* from those secretions. The *permease hypothesis* proposes that the aldosterone-induced proteins increase the number of Na^+ channels in the luminal membrane of target cells, enhancing its permeability to Na^+ . This may increase the diffusion of Na^+ through the Na^+ channels down its inward electrochemical gradient from the secretions into the respective epithelial cells. The *metabolic hypothesis* proposes that the aldosterone-induced proteins increase mitochondrial oxidation and production of ATP for use as an energy source in running the Na^+ pump in the basolateral membrane of target cells. This promotes the activity of the Na^+ pump

which actively transports Na^+ from the cell to the interstitial fluid to lower the intracellular Na^+ concentration and to enhance thereby the diffusional influx of Na^+ from the secretions into the cells across their luminal plasma membrane. This is supported by the finding that aldosterone induces the synthesis of citrate synthase and several other mitochondrial enzymes and also enhances the $\text{NADH}:\text{NAD}^+$ ratio in mitochondria, all these promoting mitochondrial ATP production. According to the *sodium pump hypothesis*, aldosterone-induced proteins may act as either enzymes or carriers for the Na^+ pump of the basolateral plasma membrane, or as an ATPase for utilizing ATP in running the Na^+ pump. This may enhance the active Na^+ transport by the latter and thereby lead to an increase in Na^+ reabsorption. However, a direct action of aldosterone on the Na^+ pump has not been demonstrated.

(b) Aldosterone increases the *secretion* of K^+ in the urine by the renal distal tubules and collecting ducts. This may result from the elimination of K^+ in exchange of the reabsorbed Na^+ due to the aldosterone-stimulated activity of the sodium pump in the basolateral cell membrane— K^+ is actively pumped into the cell from the interstitial fluid and may diffuse thence to the urine through the luminal membrane. Alternatively, aldosterone may induce proteins involved specifically either in active K^+ transport or in K^+ channels on the luminal plasma membrane.

By increasing Na^+ reabsorption, aldosterone secondarily increases the osmotic reabsorption of water from the urine.

Action of sex corticoids :

Androgenic steroids like androstenedione and dehydroepiandrosterone promote the growth and differentiation of male secondary sex characters probably by a protein anabolic effect.

Cushing's syndrome :

This disease is produced by cortisol hypersecretion from an adrenocortical tumor. Symptoms resemble those in Cushing's disease (page 604).

Aldosteronism :

In this disease, excessive amounts of aldosterone are secreted either from an adrenal aldosteronoma or owing to renin-angiotensin hyperactivity. This produces K^+ diuresis, low urinary Na^+ , hypernatremia, hypokalemia, water retention, hypertension, membrane hyperpolarization and consequent muscle paralysis.

Addison's disease :

This disease is caused by an adrenocortical atrophy either from an autoimmune, tubercular or malignant affliction of the adrenals, or from corticotropin deficiency. The resulting deficiency of corticosteroids produces hypoglycemia, reduced urinary NPN and K^+ , increased urinary Na^+ and volume, hyponatremia, low blood volume, hypotension, hyperkalemia, cardiac toxicity and arrhythmia. The fall in blood cortisol causes a compensatory rise in POMC gene transcription (page 604) and a consequent rise in the secretion of lipotropins with MSH activity, resulting in an abnormal bronze pigmentation of skin and mucus membranes.

Adrenal virilism :

A hypersecretion of androgenic corticosteroids in women, sometimes due to a mutant gene, causes an involution of female secondary sex characters and their replacement by male sex characters.

Regulation of glucocorticoids :

Cortisol secretion has a diurnal rhythm with its peak at 4-8 AM in the morning and its nadir at night. Human adrenal cortex secretes about 8-30 mg of cortisol and 1-4 mg of corticosterone in 24 hours.

Glucocorticoid secretion is stimulated by pituitary corticotropin (ACTH) which is in turn regulated by hypothalamic CRH and negative feedback effects of high blood cortisol (pages 603-604).

Regulation of mineralocorticoids :

Human adrenal cortex secretes about 100-300 μg of aldosterone in 24 hours. Its secretion rises in day hours, with activity and in erect posture.

Aldosterone secretion is directly stimulated by a rise in plasma K^+ concentration by even 0.1 mEq l^{-1} . K^+ action is mediated by an increase in the cytosolic Ca^{2+} concentration in the zona glomerulosa cells. A rise in K^+ concentration increases the activity of some enzymes for converting cholesterol to pregnenolone and corticosterone to aldosterone, and may also up-regulate the number of angiotensin receptors in glomerulosa cells, increasing their sensitivity to angiotensin. A fall in plasma K^+ decreases the sensitivity of zona glomerulosa cells to both angiotensin and low plasma Na^+ concentration by down-regulating the angiotensin receptors. *Angiotensin II* is secreted due to Na^+ depletion, hypotension, hypovolemia or a rise to erect posture. It stimulates aldosterone secretion. Its action seems to be mediated by inositol 1,4,5-triphosphate, diacylglycerols and Ca^{2+} , one or more of which may act as its second messenger. Angiotensin II increases the synthesis of aldosterone in glomerulosa cells by enhancing enzyme activities for converting cholesterol to pregnenolone and corticosterone to aldosterone. Angiotensin II can up-regulate the number of its own receptors in the glomerulosa cells to enhance their sensitivity to angiotensin. Low plasma Na^+ may also stimulate aldosterone secretion by directly increasing its synthesis and also by stimulating the renin-angiotensin system. However, the glomerulosa cells are less sensitive to Na^+ depletion than to K^+ excess—aldosterone secretion rises signi-

ficantly only when the plasma Na^+ concentration falls by about 20 mEq l^{-1} . *Corticotropin* (ACTH) seems to exert a facilitatory or permissive action for the cortical effects of high K^+ concentration or angiotensin II. ACTH administration initially increases the outputs of both aldosterone and deoxycorticosterone, but the output of aldosterone declines in a couple of days while that of deoxycorticosterone remains enhanced so long as ACTH action continues. The basal level of aldosterone secretion and its rise due to Na^+ depletion are undiminished even after hypophysectomy, but its rises due to stress, physical trauma, anxiety and surgery are largely abolished. ACTH seems to stimulate some early steps in the synthesis of deoxycorticosterone from cholesterol, but may play only a minor role in aldosterone secretion.

18.14 TESTICULAR HORMONE

Interstitial cells of Leydig in the testis secrete the male sex hormone, *testosterone*, an androgenic C_{19} steroid. [*Androgens* can develop and maintain secondary male sex characters and include testosterone, androstenedione and dehydroepiandrosterone.]

Metabolism :

Cholesterol is converted to pregnenolone in the Leydig cell mitochondria in the same way as in the adrenal cortex (page 615). This is the rate-limiting step of testosterone synthesis. Pregnenolone is then translocated to smooth endoplasmic reticulum and converted to testosterone through either a Δ^4 pathway or a Δ^5 pathway (Fig 18.23). In the Δ^4 pathway which probably predominates in man, pregnenolone is changed by 3β -hydroxysteroid dehydrogenase and $\Delta^{5,4}$ -isomerase into progesterone (Fig. 18.22). The latter is hydroxylated by 17α -hydroxylase into 17 -hydroxyprogesterone which loses its sidechain under the action of $17,20$ -lyase to give androstenedione. The latter is reduced at its C^{17}

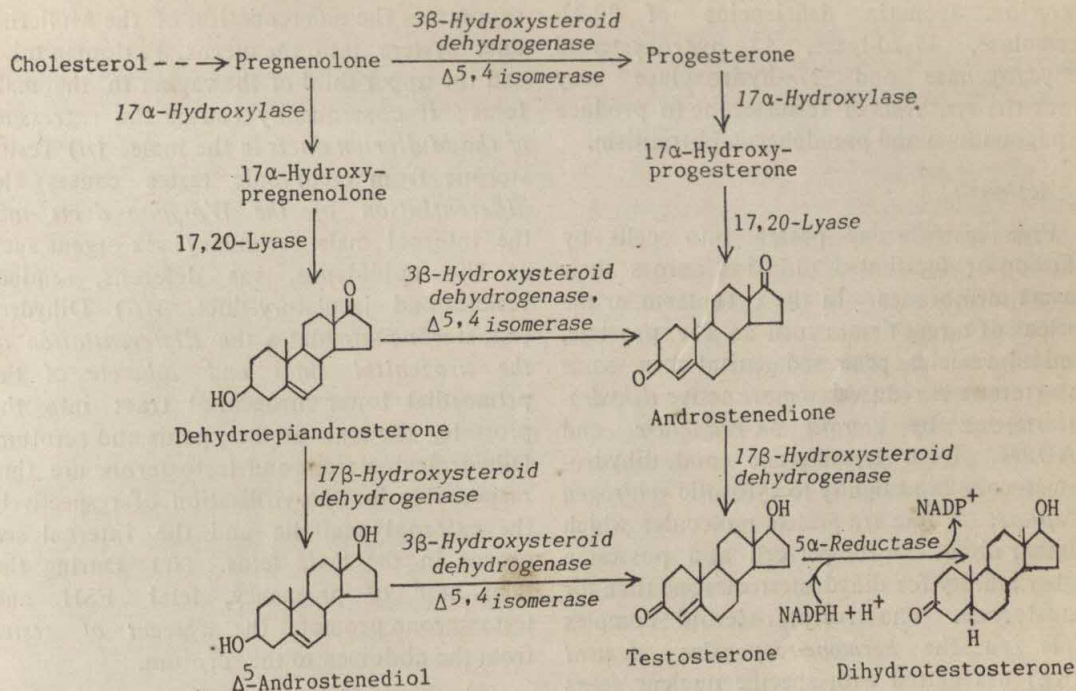


Fig. 18.23. Biosynthesis of testosterone and dihydrotestosterone.

by 17 β -hydroxysteroid dehydrogenase to form testosterone (Fig. 18.23). In the Δ^5 pathway, 17 α -hydroxylase converts pregnenolone to 17 α -hydroxypregnenolone whose sidechain is cleaved away by 17,20-lyase to give dehydroepiandrosterone (DHEA). The latter is reduced first by 17 β -hydroxysteroid dehydrogenase to Δ^5 -androstenediol which is then reduced and isomerized to testosterone by 3 β -hydroxysteroid dehydrogenase and $\Delta^5,4$ -isomerase; alternatively, DHEA is changed first by the last two enzymes to androstenedione which is then reduced to testosterone by 17 β -hydroxysteroid dehydrogenase.

To some extent in the testis, but mostly in extratesticular tissues like seminal vesicles and prostate, testosterone is reduced at its Δ^5 double-bond by steroid 5 α -reductase and NADPH to give its more potent derivative dihydrotestosterone (DHT).

Testicular androgens are catabolized

largely in the liver to 17-ketosteroids such as androsterone and etiocholanolone. Their glucuronides and sulfates as well as testosterone glucuronide are excreted in the urine.

About 4-10 mg of testosterone are secreted daily. Each dl of plasma of adult man carries about 400-1000 ng of testosterone; of this, about 86-89% is transported bound non-specifically to serum albumin, about 10% bound specifically and more tightly to a β -globulin called the sex hormone-binding globulin or testosterone-estrogen-binding globulin (SHBG or TEBG), and the remaining 1-3% (10-30 ng dl⁻¹) in the active free form which is in equilibrium with the protein-bound forms. About 0.4 mg of testosterone is daily reduced to dihydrotestosterone in testes and target tissues.

Hypogonadism (with undeveloped or involuted male secondary sex organs) results from the failure of testosterone synthesis and

secretion. Genetic deficiencies of 20,22-desmolase, 17,20-lyase, 17 β -hydroxysteroid dehydrogenase and 17 α -hydroxylase may block the synthesis of testosterone to produce hypogonadism and pseudohermaphroditism.

Actions :

Free testosterone passes into cells by diffusion or facilitated diffusion across their plasma membranes. In the cytoplasm or the nucleus of target tissues such as the prostate, seminal vesicles, penis and genital skin, some testosterone is reduced to more active *dihydrotestosterone* by *steroid 5 α -reductase* and NADPH. Both testosterone and dihydrotestosterone bind tightly to cytosolic *androgen receptors*. These are protein molecules which number about 5×10^4 per cell and possess a higher affinity for dihydrotestosterone than for testosterone. The receptor-steroid complex binds to the *hormone-responsive element* (HRE) associated with specific nuclear genes and induces the transcription of those genes (page 577). This leads to an increased synthesis of the respective proteins which produce the cellular effects.

(a) *Sexual differentiation* : The primordial genital duct system, comprising the Wolffian and Mullerian ducts, gets differentiated into either the male or the female genital tract depending on respectively the presence or absence of testicular hormones in the embryo. Preceding the *virilization* (masculinization) of the genital ducts, Leydig cells and Sertoli cells of the embryonic testis start secreting respectively *testosterone* and *anti-Mullerian hormone* (*Mullerian regression factor*). There is a simultaneous rise in *steroid 5 α -reductase* activity in those parts of the Wolffian tissue which are destined to form the prostate and male external genitalia ; this enzyme catalyzes the formation of *dihydrotestosterone* from testosterone in the embryonic Wolffian tissue.

(i) The anti-Mullerian hormone is a homodimeric glycoprotein (MW $\approx$ 70 kdal) which

suppresses the differentiation of the Mullerian duct system into the uterus, Fallopian tubes and the upper third of the vagina in the male fetus ; it consequently causes the *regression of the Mullerian ducts* in the male. (ii) Testosterone from embryonic testes causes the *differentiation of the Wolffian ducts* into the internal male secondary sex organs such as the epididymis, vas deferens, seminal vesicles and ejaculatory duct. (iii) Dihydrotestosterone stimulates the *differentiation of the urogenital sinus and tubercle* of the primordial lower urogenital tract into the prostate, the male urethra, penis and scrotum. Dihydrotestosterone and testosterone are thus responsible for the virilization of respectively the external genitalia and the internal sex organs in the male fetus. (iv) During the later half of pregnancy, fetal FSH and testosterone promote the *descent of testes* from the abdomen to the scrotum.

(b) *Spermatogenesis* : Testosterone stimulates spermatogenesis by a paracrine action on seminiferous tubules in the sexually mature animal. Both FSH and testosterone stimulate the Sertoli cells to secrete an *androgen binding protein* (ABP) into the lumen of seminiferous tubules. ABP binds and locally concentrates testosterone reaching the tubules by diffusion from the neighbouring interstitial tissue. This enables testosterone to promote spermatogenesis, particularly the formation of spermatogonia and the meiosis of spermatocytes. FSH is essential for this spermatogenic effect ; testosterone administration fails to stimulate spermatogenesis in hypophysectomized or immature animals.

(c) *Maturation of male secondary sex tissues* : The pubertal maturation and the postpubertal maintenance of the male secondary sex tissues depend on particularly the action of dihydrotestosterone produced in those tissues from testosterone. These effects result from the steroid-induced stimulation of gene transcription and protein synthesis in

those tissues. Dihydrotestosterone stimulates the growth, development and functions of *male secondary sex organs* such as the prostate and penis as also the development of *accessory male sex characters* like beards, moustaches, male pubic hair pattern and pigmentation of external genitalia skin. The growth of the larynx and the deepening of the voice depend more on testosterone itself. Castration before puberty keeps these tissues permanently undeveloped and infantile while castration after puberty causes their rapid involution to the prepubertal form with loss of function. Enzymes whose activities are enhanced by testicular hormones include DNA polymerase, thymidine kinase and RNA polymerase in the prostate, and aldose reductase and ketose reductase in seminal vesicles.

(d) *Anabolic and growth effects*: Testosterone and dihydrotestosterone are protein-anabolic and growth-promoting steroids. They stimulate the retention of N , Ca^{2+} , PO_4^{3-} , Na^+ and K^+ , and the synthesis of DNA, RNA, proteins and membrane phosphoglycerides. Testosterone increases cellular mitosis and growth of many somatic tissues such as epiphyseal cartilages, long bones, skeletal muscles, kidneys, liver and skin. Testosterone also promotes the closure of bony epiphyses.

(e) *Actions on brain*: Testosterone, at least partly through its conversion to dihydrotestosterone in the brain neurons, influences the growth and development of the fetal and infant brain and develops the masculine psychology and sexual behaviour at puberty. Besides, testosterone causes feedback inhibitions of hypothalamic GnRH and hypophyseal gonadotropin secretions.

Cryptorchidism :

In this disease, testis fails to descend into the scrotum. Seminiferous tubules degenerate due to abdominal heat and spermatogenesis fails. But interstitial tissue, blood testo-

sterone level and male secondary sex tissues are normal.

Male pseudohermaphroditism :

This is characterized by the possession of male XY genotype but different degrees of female phenotype by a patient.

(a) *Testicular feminization*: This is a genetic failure of testosterone action resulting from an inherited abnormality or absence of the cytosolic androgen receptor in the male secondary sex tissues, anterior pituitary and seminiferous tubules. The patient has a male (XY) genotype with undescended testes, a high blood LH level in absence of the feedback inhibition of LH secretion by testosterone, and consequently a high blood testosterone level. But spermatogenesis is impaired due to the failure of testosterone action on seminiferous tubules. Epididymis, vas deferens, seminal vesicles, prostate, ejaculatory ducts and penis are all absent due to the failure of embryonic differentiation of the Wolffian duct system. Thus, the patient has female external genitalia consisting of a short blind vagina. But as the Mullerian regression hormone is still secreted by embryonic testes, the Mullerian duct system undergoes regression and the uterus, Fallopian tubes and the upper third of the vagina are absent.

(b) *Genetic error of 5 α -reductase*: A genetic deficiency of 5 α -reductase causes a failure in converting testosterone to dihydrotestosterone. In absence of dihydrotestosterone, penis, scrotum and male pattern of hair distribution are absent, the prostate and sebaceous glands remain abnormally small, and the patient has female external genitalia with a short, blind vaginal pouch. The uterus, Fallopian tubes and the upper third of the vagina are absent because the Mullerian duct system has undergone regression due to the Mullerian regression hormone from testes. The testes descend to

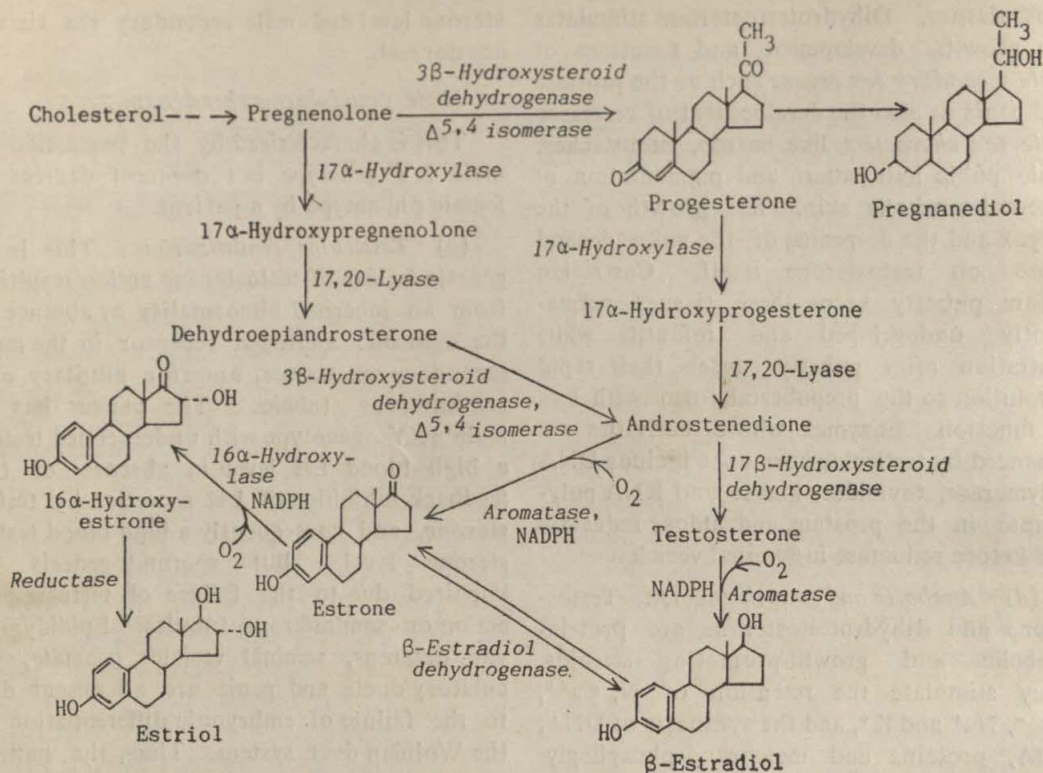


Fig. 18.24. Metabolism of ovarian steroids.

labial locations. Spermatogenesis and testosterone secretion take place. Due to the action of testosterone itself, internal male sex organs such as epididymis, vas deferens and seminal vesicles develop; puberty changes like skeletal and muscular growth, masculine behaviour and deepening of voice may also occur.

Regulation :

Testosterone secretion is regulated by LH of the anterior pituitary. In turn, a high blood testosterone level causes the feedback inhibition of LH (pages 600, 602).

18.15 OVARIAN HORMONES

Structure and metabolism :

The granulosa cells of ovarian Graafian follicles secrete *estrogens*. **17 β -estradiol** (E_2),

estrone (E_1) and *estriol* are the principal estrogens with biological potencies in a steeply descending order. Estrogens are C_{18} steroids, lacking the C^{10} -methyl substituent and possessing an aromatic A ring and a C^3 -OH group (Fig. 18.24). The cells of the corpus luteum secrete *progesterone*, a C_{21} steroid (Fig. 18.22).

Ovarian steroidogenic cells synthesize progesterone from cholesterol by a pathway identical with that in the adrenal cortex (Fig. 18.22). Luteal cells secrete most of this progesterone. But in the Graafian follicle, theca interna cells change both pregnenolone and progesterone to androstenedione and testosterone as in the testis (Fig. 18.23). In the adjacent granulosa cells of the follicle, testosterone is next converted to 17 β -estradiol through the aromatization of its A ring by a

microsomal enzyme system, called *aromatase* (Fig. 18.24). The latter has a cytochrome P_{450} monooxygenase as a component. It catalyzes three successive hydroxylations of testosterone, two at the C^{19} -methyl group and one at the C^2 , using one NADPH as the electron donor and one O_2 molecule at each step. The final hydroxylated product loses its C^{18} non-enzymatically and changes into estradiol. Androstenedione may be converted directly by aromatase into estrone. Besides, the liver, adipose tissue, muscles, hair follicles and other extraovarian tissues may also aromatize circulating dehydroepiandrosterone, androstenedione and testosterone into estradiol and estrone in considerable amounts in both sexes.

In extraovarian tissues such as the liver and placenta, *17 β -estradiol dehydrogenase* catalyzes the interconversion of estradiol and estrone; microsomal *16 α -hydroxylase* changes estrone to 16 α -hydroxyestrone which is reduced to estriol by a *reductase*. In the brain, estradiol is changed to very weak catecholestrogens by a microsomal *estradiol 2-hydroxylase*. Both the hydroxylases possess cytochrome P_{450} monooxygenase as a component and use NADPH and molecular O_2 .

Estrone and estriol are the principal metabolic products of estradiol. Along with catecholestrogens, they are excreted mainly as glucuronides and sulfates in the bile and urine. *Pregnandiol* is the principal metabolite of progesterone. It is formed chiefly in the liver and excreted in the urine as glucuronide and sulfate.

Actions :

After entering the target cells in the uterus, vagina, Fallopian tubes, mammary glands and brain, estrogens and progesterone bind respectively to estrogen and progesterone receptors in the cytosol. The receptor-steroid complexes bind to the hormone-responsive elements (HRE) associated with specific nuclear genes and consequently increase the

transcription of those genes. This results in the increased synthesis of specific proteins and enzymes, producing the ultimate effects.

(a) *Puberty changes*: Estrogens stimulate the growth and development of *female secondary sex organs* such as the uterus, Fallopian tubes, vagina and breasts at puberty, and maintain them till menopause. They also develop and maintain the *female accessory sex characters* like the female patterns of pubic hair and body fat deposition. They also promote the *growth of somatic tissues* like muscles and bones; estradiol retains nitrogen, Ca^{2+} and phosphates in the body, increases the synthesis of DNA, RNA and proteins in tissue cells and consequently enhances the number and size of the cells. Estradiol *prevents osteoporosis* which is frequently seen after the menopausal decline in estrogen secretion. Estrogens also stimulate the *closure of bony epiphyses*. Acting on the brain, estrogens promote the pubertal development of *feminine personality and sexual behaviour*.

(b) *Menstrual and estrous cycle changes*: Estrogens control the changes in the female secondary sex organs in the first half of menstrual and estrous cycles. Thus, estrogens stimulate endometrial proliferation, hypertrophy and elongation of uterine glands, rhythmic motility of uterine muscles, number and movements of cilia in Fallopian tubes, vascularization of uterus, and proliferation and cornification of vaginal epithelium. Near the middle of the cycle, either the positive feedback effect of the peak blood estradiol level or a sudden fall in that level stimulates an upsurge in the LH secretion; this leads to ovulation and corpus luteum formation.

Progesterone regulates the changes in the later half of the cycle. Thus, it causes further growth of uterine endometrium and glands, secretion of uterine glands, inhibition of uterine motility and some regression of vaginal epithelium. It also decreases the peripheral

blood flow to reduce cutaneous heat loss and consequently raises the body temperature.

At the end of the menstrual cycle, a fall in blood ovarian steroids causes a breakdown of the thickened endometrium, resulting in menstruation.

(c) *Pregnancy changes* : Estrogens promote the hypertrophy of uterine muscles, growth of the mammary duct system and fluid retention in the body. Progesterone causes the implantation of the fertilized ovum, placenta formation, growth changes in the female reproductive tract, suppression of ovulation and estrus, decreased tone and contraction of uterine muscles, and development of lobulo-alveolar system of mammary glands. High blood progesterone inhibits lactation during pregnancy.

Estrogens and progesterone *influence each other's actions*. Estradiol up-regulates both estrogen receptors and progesterone receptors in target cells and thereby augments the actions of both hormones. Progesterone induces the synthesis of 17β -estradiol dehydrogenase in the extraovarian tissues like the liver and thereby enhances the conversion of estradiol to less active estrone.

Some actions of ovarian hormones are not mediated through cytosolic receptors which interact with nuclear chromatin.

Relaxin :

The corpus luteum synthesizes and secretes this basic peptide (MW $\approx$ 5.5 kD) in the last part of pregnancy. It softens the connective tissue of pelvic ligaments and symphysis pubis by promoting the depolymerization of matrix mucoproteins and water imbibition. This enables a separation of the symphysis pubis and a dilatation and softening of uterine cervix, facilitating childbirth. Porcine relaxin is made of two peptide chains of 22 and 26 amino acid residues. It has two S-S bonds linking its two chains, and one intrachain S-S bond in the A chain.

Secretion and regulation :

About 0.1-0.2 mg of estrogens are secreted daily in women ; at the time of ovulation, they average about 0.5 mg day^{-1} . The normal serum level of estradiol amounts to about $25\text{-}70 \text{ pg ml}^{-1}$ during the first 10 days of menstrual cycle, $50\text{-}300 \text{ pg}$ during the next 10 days and $70\text{-}150 \text{ pg}$ during the last 10 days. Progesterone is secreted at the rate of about 2 mg day^{-1} in the follicular phase and about 25 mg day^{-1} in the luteal phase of the menstrual cycle. Serum progesterone concentrations normally amount to $0.2\text{-}1.5 \text{ ng ml}^{-1}$ in the follicular phase and $6\text{-}30 \text{ ng ml}^{-1}$ in the luteal phase (Fig. 18.25).

About 1-3% of the plasma estradiol and estrone circulate in active free forms which are in equilibrium with the protein-bound forms. Much larger amounts of estrogens are carried, tightly and specifically bound to the β -globulin called the *sex hormone-binding globulin* (SHBG). Still greater amounts are transported, bound less tightly and non-specifically with serum albumin. Active and free progesterone constitutes 1-5% of the total serum progesterone. About 40% of the latter is carried, tightly bound to the *cortisol-binding globulin* ; the rest remains bound less tightly to albumin, orosomucoid and other plasma proteins.

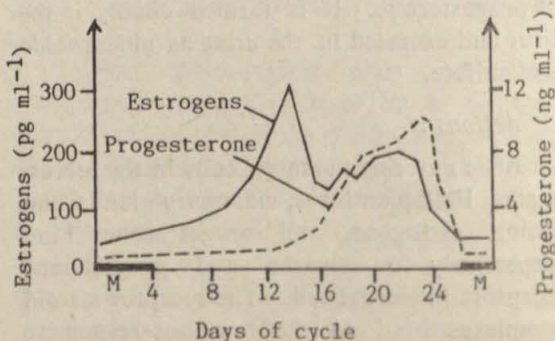


Fig. 18.25. Blood levels of ovarian hormones in the menstrual cycle. M : menstruation.

The secretion of ovarian steroids is regulated by pituitary gonadotropins which are under a complex feedback control of blood ovarian hormones (pages 600-602).

18.16 PLACENTAL HORMONES

Human placenta secretes several peptide hormones (e.g., human chorionic gonadotropin and chorionic somatomammotropin) and the ovarian steroid hormones.

Human chorionic gonadotropin

This is a glycoprotein. Its apoprotein part is a heterodimer of noncovalently linked α and β subunits. The α chain is identical with the α chains of human FSH, LH and TSH. It is made of 92 amino acids and carries two asparagine-linked oligosaccharides. The β chain of human chorionic gonadotropin (hCG) is made of 145 amino acids and carries two asparagine-linked and four serine-linked oligosaccharides.

hCG starts being secreted by the syncytiotrophoblast of chorionic villi within 2 weeks of fertilization. The blood and urinary levels of hCG rise rapidly in the first few weeks; the plasma hCG reaches its peak of about 100 IU ml⁻¹ by the 10th week of pregnancy, declines slowly thereafter to about 15-20 IU ml⁻¹ by the 17th week, and continues at the low level for a few days after parturition due to its long half-life.

Actions :

hCG binds with specific membrane receptors on the target cells in ovaries and testes, and thereby activates adenylate cyclase (page 578). The latter increases cAMP in the cytoplasm. cAMP acts as the second messenger to produce the biological effects.

(a) Like LH, hCG stimulates the enlargement and secretion of corpus luteum (*luteotropic effect*). hCG mainly functions in maintaining a secretory corpus luteum, particularly in the first trimester of pregnancy.

(b) hCG resembles LH in stimulating the growth of interstitial tissue and *secretion of testosterone* in the embryonic testis. This helps in the virilization of the reproductive system in the male embryo.

(c) hCG has a weak *follicle-stimulating effect*, but cannot maintain the structure and function of ovary after hypophysectomy.

Pregnancy tests :

Urinary hCG is the basis of pregnancy tests within the first 30 days of gestation.

(a) *Ascheim-Zondek test* : The urine of the patient is injected into immature female rats or mice. Subsequent appearance of blood spots in their ovaries indicates the presence of hCG in the urine, signifying pregnancy.

(b) *Anti-hCG serum slide test* : The patient's urine is mixed on a glass slide with anti-hCG serum from sensitized rabbits. hCG-coated latex particles are then mixed with that mixture. The final mixture is examined within 2 minutes for agglutination of the hCG-coated latex particles. Absence of agglutination indicates pregnancy, because hCG of the pregnant patient's urine binds the antibodies of the anti-hCG serum, leaving no antibody for agglutinating the latex particles.

Chorionic somatomammotropin

Chorionic somatomammotropin (CS) or *placental lactogen* belongs to the same group of protein hormones as the growth hormone and prolactin of anterior pituitary. There is 85% homology in amino acid sequence between CS (MW $\approx$ 21 kdal) and GH of humans.

Chorionic somatomammotropin is secreted by the syncytiotrophoblast from about the 2nd week of pregnancy, rises slowly in the maternal blood to reach a peak of about 18 μ g ml⁻¹ by the 35th-38th week and continues subsequently at about the same level.

Actions :

CS has some effects like GH and prolactin.

(a) It stimulates the growth, enlargement and secretion of the corpus luteum (*luteotropic effect*).

(b) It may act with ovarian and pituitary hormones to stimulate the lobuloalveolar growth of mammary glands during pregnancy (*mammotropic effect*). It may also stimulate lactation (*lactogenic effect*).

(c) It may promote some growth of maternal tissues (*somatotropic effect*). It also stimulates retention of Ca^{2+} , phosphates and nitrogen (*anabolic effect*) for utilization in fetal and maternal tissue growth.

(d) It is *anti-insulin* in effect and may decrease glucose utilization and carbohydrate tolerance to produce hyperglycemia.

(e) It *enhances lipolysis* in the adipose tissue to raise the serum free fatty acid level.

Ovarian steroids

Placenta is the major source of ovarian steroids in the advanced stage of gestation. From about the 5th week, placental trophoblast synthesizes estrogens from dehydroepiandrosterone and its 16α -hydroxy derivative collected from fetal and maternal bloods. It secretes mainly estrinol.

Placental trophoblast collects low-density lipoproteins from the maternal plasma by adsorptive pinocytosis, hydrolyzes them to release cholesterol and synthesizes large amounts of progesterone from it.

18.17 PANCREATIC HORMONES

Three major peptide hormones, viz, insulin, glucagon and somatostatin, are secreted by α , β and δ cells of pancreatic islets, respectively. (For *somatostatin*, see page 590).

Glucagon

It is a monomeric peptide (MW 3485) made of 29 amino acids, but devoid of cysteine (Fig. 18.26). It has close structural similarities with secretin, GIP and VIP.

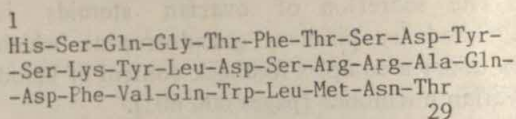


Fig. 18.26. Amino acid sequence of human glucagon.

Glucagon is formed by the post-translational modification of a large, inactive *proglucagon* molecule synthesized in pancreatic α cells. Trypsin-like and carboxypeptidase B-like peptidases of α cells hydrolyze proglucagon from both its N- and C-terminal ends to yield glucagon and inactive peptides.

Glucagon is catabolized mainly in the liver by proteases including a dipeptidyl aminopeptidase which hydrolyzes the $\text{Ser}^2\text{—Gln}^3$ peptide bond to liberate histidylserine from its N-terminal end.

Actions :

Glucagon binds to specific receptors on the plasma membranes of hepatocytes and adipocytes. This activates adenylate cyclase which catalyzes the formation of cAMP in these cells. cAMP is the principal second messenger for glucagon. It allosterically activates cAMP-dependent protein kinases which phosphorylate specific cellular proteins (enzymes) to increase or decrease their activities. cAMP also induces the synthesis of specific enzymes by increasing the transcription of their genes; this is comparable to the induction of lac operon in *E. coli* by the binding of cAMP to a *catabolite gene activator protein* (page 101).

Glucagon is secreted when a short supply of fuel substances decreases the blood levels of glucose and free fatty acids. The principal physiological role of glucagon is to prevent hypoglycemia and to maintain fuel supply to the tissues by enhancing both blood sugar and blood fatty acid levels. Together with catecholamines, growth hormone and cortisol, it counterbalances the hypoglycemic and antilipolytic effects of insulin.

(a) *Hyperglycemic action*: Glucagon was initially called the *hyperglycemia glucosuria factor* (HG factor) because it can raise the blood sugar, lower the liver glycogen and even cause glucosuria. It produces hyperglycemia in several ways. (i) Glucagon increases glycogenolysis in the liver. First, the glucagon-induced rise in cAMP activates a protein kinase which phosphorylates and activates glycogen phosphorylase kinase; the latter in turn phosphorylates and activates glycogen phosphorylase, the rate-limiting enzyme for glycogenolysis. However, glucagon does not activate muscle glycogen phosphorylase. Secondly, glucagon prevents the dephosphorylation and inactivation of glycogen phosphorylase by protein phosphatase I because the cAMP-dependent protein kinase phosphorylates and activates phosphatase inhibitor I which inhibits the protein phosphatase. Thirdly, glucagon induces the synthesis of glucose 6-phosphatase, the final enzyme for glycogenolysis. (ii) Glucagon increases gluconeogenesis in the liver. First, it induces the synthesis of fructose 1,6-bisphosphatase, pyruvate carboxylase and PEP carboxykinase (PEPCK), all key enzymes for gluconeogenesis. Indeed, the glucagon-stimulated rise in cAMP increases the transcription of the PEPCK gene, leading to a rise in PEPCK synthesis. Secondly, glucagon expands the hepatic pool of free gluconeogenic amino acids by increasing proteolysis in the liver and by reducing hepatic protein synthesis through a delay at the elongation or termination step of ribosomal translation. (iii) Glucagon decreases glycogenesis in the liver through the cAMP-mediated activation of protein kinase which phosphorylates and inactivates glycogen synthase, the rate-limiting enzyme for glycogenesis. (iv) Glucagon inhibits glucose utilization by causing the cAMP-mediated phosphorylation and inactivation of pyruvate kinase and pyruvate dehydrogenase.

(b) *Lipolytic and ketogenic actions*: In

adipocytes, the glucagon-stimulated rise in cAMP activates a protein kinase which phosphorylates and activates triacylglycerol lipase, the rate-limiting enzyme for adipose tissue lipolysis. Glucagon consequently increases lipolysis in adipocytes and mobilizes the released fatty acids into the blood, leading to increased β -oxidation and ketosis. Thyroid hormones augment the lipolytic action of glucagon by up-regulating the number of glucagon receptors on adipocytes.

(c) *Anti-lipogenic action*: Glucagon reduces fatty acid biosynthesis in two ways.

(i) Glucagon-stimulated rise in cAMP activates a protein kinase which phosphorylates and inactivates acetyl-CoA carboxylase, the rate-limiting enzyme for fatty acid synthesis. (ii) By promoting adipose tissue lipolysis and mobilization of fatty acids, glucagon raises the cytoplasmic acyl-CoA level in both hepatocytes and adipocytes. This inhibits acetyl-CoA carboxylase.

Regulation:

Low blood level of either glucose or free fatty acids stimulates glucagon secretion. Starvation increases glucagon secretion by causing hypoglycemia. Rise in blood levels of most gluconeogenic amino acids, particularly arginine and alanine, stimulates glucagon secretion—its significance lies in the gluconeogenic role of glucagon. Rise in serum Ca^{2+} concentration is a potent stimulus for glucagon secretion. Adrenergic sympathetic neurons are involved in the stimulation of glucagon secretion during exercise and stress. Indeed, both cholinergic parasympathetics and adrenergic sympathetics may enhance glucagon secretion because pancreatic α cells carry both muscarinic receptors for acetylcholine and β -adrenergic receptors. Pancreatic somatostatin and insulin inhibit glucagon secretion by paracrine action on the α cells.

Insulin

Insulin is a heterodimeric protein (MW

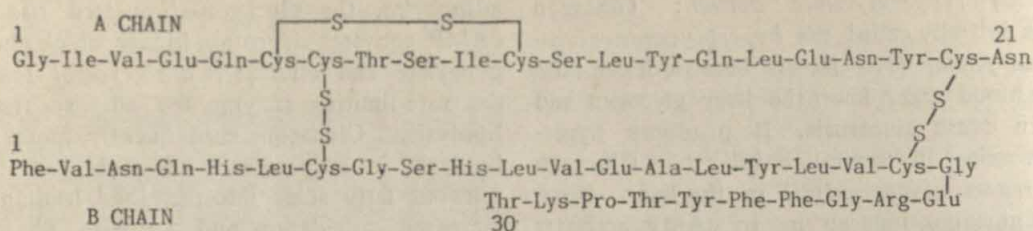


Fig. 18.27. Amino acid sequence of human insulin.

5734), composed of an A chain having 21 amino acid residues and a B chain with 30 amino acid residues. There are two inter-chain S-S linkages joining the Cys⁷ and Cys²⁰ of the A chain with respectively the Cys⁷ and Cys¹⁹ of the B chain (Fig. 18.27). The A chain carries an intrachain S-S linkage between its Cys⁶ and Cys¹¹ residues.

Synthesis :

Polysomes, attached to the membrane of rough endoplasmic reticulum (RER) of pancreatic β cells, translate *preproinsulin* (MW $\approx$ 11.5 kdal) composed of 109 amino acid residues in man. It is translocated into the lumen of RER cisternae and its N-terminal *signal (leader) sequence* of 23 amino acids is hydrolyzed away by a *signal peptidase* of the RER membrane to produce *proinsulin* (MW 9 kdal). The latter has 86 amino acid residues in humans. Small transfer vesicles, containing proinsulin, are pinched off from endoplasmic reticulum and go to fuse with the Golgi cisternae, delivering proinsulin to them. In the Golgi cisternae, a *trypsin-like protease* hydrolyzes about 95% of the proinsulin at two sites, each at the C-terminal end of two sequential basic amino acid residues, to yield two intermediates destined to form respectively insulin and an inactive *connecting or C peptide* (Fig. 18.28). A *carboxypeptidase B-like enzyme* then hydrolyzes the C-terminal peptide bonds in the two intermediates to release two C-terminal basic amino acids from each of them and thereby change them to

insulin and C peptide. The latter has 31 amino acid residues in humans.

Condensing vacuoles are pinched off from the Golgi cisternae with equimolar amounts of insulin and C peptide in their lumen. Insulin molecules in their lumen form dimers through hydrogen bonding between the peptide groups of Phe²⁴ and Tyr²⁶ residues of their B chains. With the progressive concentration of their contents, condensing vacuoles change into secretory granules. In them, insulin forms crystalloid-like hexamers with two Zn²⁺ each while C peptides occur in the fluid-filled spaces surrounding the crystalloids. Insulin is released from the β cell by exocytosis or emiocytosis, through the fusion of the granule membrane with the plasma membrane.

Catabolism :

Insulin has a half life of about 3-10 minutes in the plasma. After insulin has bound to insulin receptors on the target cell membrane, the receptor-insulin complex is aggregated to form patches on the membrane and then internalized into the cell by adsorptive pinocytosis. Insulin is then rapidly hydrolyzed and inactivated by lysosomal SH-dependent *insulin-specific protease*. Insulin is almost totally inactivated by the removal of only the C-terminal Asn residue of its A chain. A *glutathione-insulin transhydrogenase* uses glutathione as the hydrogen donor for the reductive cleavage of S-S linkages in insulin to release its A and B chains; these

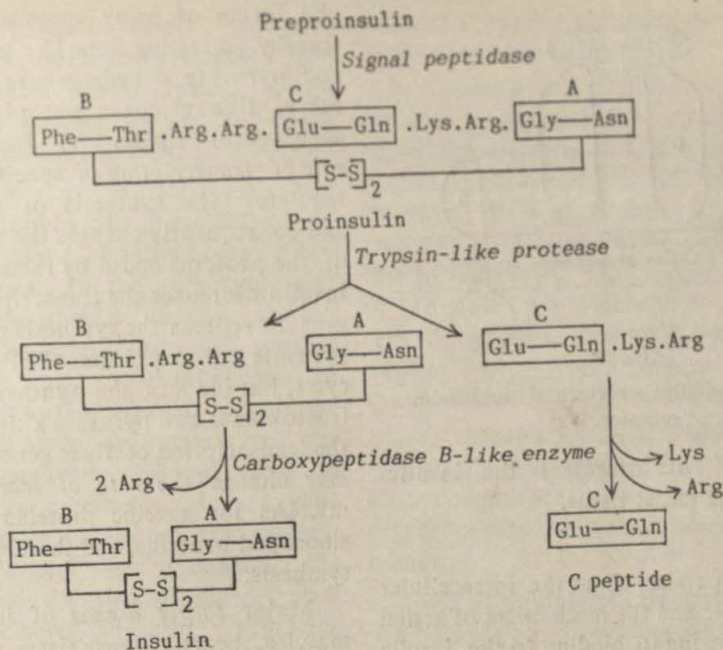


Fig. 18.28. Cleavage of human preproinsulin to insulin.

are subsequently hydrolyzed by a protease called *insulinase*, which cannot hydrolyze insulin itself. Liver, kidneys, skeletal muscles and placenta are important sites of insulin catabolism.

Insulin receptor :

Insulin acts by binding to specific trans-membrane integral glycoproteins, called insulin receptors, of the target cell membrane. They number about 15-60 per μm^2 of the membrane, have a half-life of 6-12 hrs, and are down-regulated by circulating insulin. Each insulin receptor consists of two α and two β peptide chains all glycosylated. The heavy α chains (135 kdal) project extracellularly, are interlinked by an S-S linkage, possess cysteine-rich domains constituting the *insulin-binding site*, and are each linked by an S-S linkage to a β chain (Fig. 18.29). The light β chains (95 kdal) act as *signal transducers*. Each β chain has its N-terminal third on the outer

surface of the membrane, a narrow, hydrophobic, 23-amino-acid domain spanning the membrane, and C-terminal two-thirds possessing *tyrosine kinase* and ATP-binding activities and projecting from the cytoplasmic surface of the membrane. Binding of insulin to the receptor stimulates its tyrosine kinase activity by increasing the V_{max} of that enzyme. Tyrosine kinase is a specific type of protein kinase; unlike the cAMP-dependent protein kinases which phosphorylate the OH groups of serine or threonine residues in their substrates, tyrosine kinase phosphorylates the phenolic OH group of tyrosine residues in specific proteins, including that of a tyrosine in the β chain of the insulin receptor itself, to modulate their activities: $\text{ATP} + \text{tyrosine-protein} \rightarrow \text{ADP} + \text{phosphotyrosine-protein}$.

A high blood insulin level *down-regulates*, i.e., decreases the number of insulin receptors on target cell membrane, probably through the internalization of the receptor-insulin complex

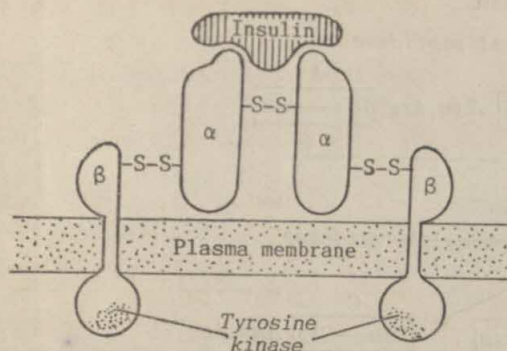


Fig. 18.29. Tentative structure of the insulin receptor.

into the cell. This decreases the insulin-sensitivity of the target tissue.

Action :

Uncertainty persists about the intracellular second messenger and the mechanism of action of insulin, following its binding to the insulin receptor. Various mechanisms have been proposed : (i) The binding of insulin to its receptor enhances the V_{max} of tyrosine kinase activity of the latter. Tyrosine kinase in turn phosphorylates tyrosine residues of specific proteins, leading to changes in enzyme activities, membrane constituent synthesis and membrane properties. (ii) The receptor-insulin interaction may activate the membrane *guanylate cyclase* which catalyzes the formation of cGMP. The latter may act as a second messenger to activate *cGMP-dependent protein kinases* (page 581). These phosphorylate some enzymes to modulate their activities. (iii) Insulin promotes the phosphorylation of *cAMP phosphodiesterase* to lower its K_m . The enzyme hydrolyzes cAMP and decreases its cellular concentration. The consequent fall in the activities of *cAMP-dependent protein kinase* reduces the phosphorylation of specific enzymes. (iv) Independent of cAMP, insulin activates *phosphoprotein phosphatases* which dephosphorylate specific phosphoproteins. In the last two ways, insulin increases the dephos-

pho forms of many enzymes in the cell, thereby activating some like glycogen synthase and pyruvate dehydrogenase and inhibiting others like glycogen phosphorylase and triacylglycerol lipase. (v) Insulin affects the rate of transcription of specific genes, thereby regulates the synthesis of specific mRNAs and consequently changes the rate of synthesis of the proteins coded by them. For example, insulin decreases the transcription of PEPCK gene to repress the synthesis of phosphoenolpyruvate carboxykinase (PEPCK) in hepatocytes, but induces the synthesis of phosphofructokinase and pyruvate kinase by enhancing the transcription of their genes. (vi) Insulin may change the rate of translation of the mRNAs for specific proteins including some ribosomal proteins, and thereby affect protein synthesis.

Major target organs of insulin are the muscles, heart, adipose tissue and liver. Erythrocytes and gastrointestinal and renal tubular epithelia are generally unresponsive to insulin.

(A) *Actions on carbohydrate metabolism* : Insulin increases the utilization of glucose in energy production and lipogenesis. It also decreases glucose formation from glycogen as well as noncarbohydrates, and indirectly enhances carbohydrate storage in tissues. All these effects serve to lower the blood glucose. Insulin deficiency produces *diabetes mellitus* with hyperglycemia, glucosuria, low RQ indicating reduced carbohydrate oxidation, increased fat oxidation and ketosis. *Experimental diabetes* has been produced in animals by total pancreatectomy, subtotal pancreatectomy followed by GH or T_4 injections, injections of anti-insulin antibodies, and administration of alloxan or streptozotocin which may selectively destroy β -cells. *Hyperinsulinism*, caused by an insulin-secreting β -cell tumor, produces severe hypoglycemia leading to the depression of higher cortical centres and even a consequent loss

of consciousness (*insulin shock*). Injection of a high dose of insulin also produces hypoglycemia and insulin shock. Insulin affects the carbohydrate metabolism and the blood sugar level in the following ways.

(i) Insulin increases glucose uptake from the extracellular fluid by the muscles, adipocytes, mammary glands, lens and many other extrahepatic tissues. In the adipocytes and probably other extrahepatic tissues, insulin probably stimulates translocation of glucose transporters from their intracellular pool in Golgi cisternae to the plasma membrane where they participate as carriers in the facilitated diffusion of D-glucose and D-galactose across the membrane. Insulin thus increases the number of glucose transporters in the membrane and thereby enhances the V_{max} of facilitated diffusion of glucose into these cells. However, insulin promotes neither such recruitment of glucose transporters nor the facilitated diffusion of glucose in the hepatic, intestinal and renal cells. Nevertheless, it increases the hepatic uptake of glucose, because it induces the synthesis of glucokinase which phosphorylates glucose into glucose 6-phosphate in hepatocytes, thereby lowering the intracellular concentration of glucose and enhancing its simple diffusion down the inward concentration gradient.

(ii) Insulin enhances glycolysis in muscles, liver and many other tissues, because it induces the synthesis of phosphofructokinase and pyruvate kinase of that pathway by enhancing the transcription of their genes and also activates pyruvate kinase by increasing its dephosphorylation.

(iii) Insulin may enhance the aerobic metabolism of pyruvate because it causes dephosphorylation of pyruvate dehydrogenase to its active form.

(iv) It may decrease glycogenolysis by repressing glucose 6-phosphatase and by

dephosphorylating glycogen phosphorylase to its inactive form.

(v) Insulin reduces gluconeogenesis, mainly because it represses the synthesis of PEP carboxykinase, the rate-limiting enzyme of this pathway, by lowering the transcription rate of its gene. Besides, insulin dephosphorylates fructose 2,6-bisphosphatase to inactivate it; this increases the intracellular concentration of fructose 2,6-bisphosphate which allosterically inhibits fructose 1,6-bisphosphatase, another key gluconeogenic enzyme, and thereby reduces gluconeogenesis.

(vi) Insulin stimulates NADPH formation by inducing the synthesis of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of the pentose phosphate pathway.

(vii) It stimulates glycogenesis in the liver and muscles by promoting dephosphorylation of glycogen synthase, the rate-limiting enzyme, and thereby activating it. Besides, insulin also induces glucokinase, the first enzyme in hepatic glycogenesis.

(B) *Actions on lipid metabolism*: Insulin exerts its anabolic actions on fats in the following ways.

(i) It decreases lipolysis in adipocytes because it activates phosphoprotein phosphatase for dephosphorylating the triacylglycerol lipase to its inactive form, and also activates cAMP phosphodiesterase which degrades cAMP to prevent phosphorylation and re-activation of triacylglycerol lipase. Insulin consequently lowers the blood level of free fatty acids; this results in the decline in β -oxidation and ketone body formation in the liver. Diabetes abnormally increases the lipase activity and the plasma FFA level.

(ii) Insulin stimulates the synthesis of fatty acids and triacylglycerols (*lipogenesis*) in several ways: (a) Probably insulin induces the synthesis of acetyl-CoA carboxylase and fatty acid synthase, the cytoplasmic

enzymes for fatty acid biosynthesis. (b) It activates acetyl-CoA carboxylase through its dephosphorylation. (c) Insulin lowers the circulating level of acyl-CoA by reducing adipose tissue lipolysis and consequently prevents acyl-CoA from inhibiting acetyl-CoA carboxylase. (d) It induces the synthesis of ATP-citrate lyase to increase the cytoplasmic cleavage of citrate to acetyl-CoA, the substrate for acetyl-CoA carboxylase. (e) Insulin promotes dephosphorylation of pyruvate dehydrogenase to its active form which enhances the formation of acetyl-CoA from pyruvate. (f) It induces the synthesis of malic enzyme, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase which generate NADPH for fatty acid biosynthesis. (g) Insulin induces the synthesis of lipoprotein lipase in adipose tissue; this enzyme hydrolyzes triacylglycerols of circulating VLDL and chylomicrons to release fatty acids which are taken up by adipocytes and used in synthesizing triacylglycerols for storage. (h) By promoting glucose uptake by adipocytes, insulin increases its conversion to fatty acids and glycerol 3-phosphate, both of which are utilized in triacylglycerol synthesis.

(C) *Actions on protein metabolism*: Insulin has anabolic actions on proteins. (i) It promotes amino acid uptake by the tissue cells by enhancing the rate of synthesis of membrane transporters for amino acids. (ii) In the liver, muscles, heart, mammary glands, adipose tissue, intestinal and reproductive tracts, adrenal cortex, anterior pituitary and lymphoid tissues, insulin affects the synthesis of many enzymes, structural proteins, carrier proteins (e.g., albumin and some globulins), secretory proteins (e.g., casein and some enzymes like amylase), and even some peptide hormones like growth hormone. Insulin changes the rates of protein synthesis by providing more amino acids in the cell, by affecting the rate of specific gene transcriptions, by regulating specific mRNA syntheses and by

affecting the ribosomal translation of specific mRNAs into proteins. (iii) Insulin regulates ribosomal translation of proteins in two major ways. It increases the synthesis of polyamines involved in rRNA synthesis, by inducing ornithine decarboxylase, the rate-limiting enzyme for polyamine formation. Secondly, insulin modulates ribosomal activity by causing the phosphorylation of the S6 protein component of 40S ribosomal subunit. (iv) Insulin decreases proteolysis, intracellular catabolism of amino acids and urea formation. In diabetes, glutamate-pyruvate transaminase and all urea cycle enzymes show heightened activities in the liver.

(D) *Actions on growth and cell replication*: Insulin stimulates cell proliferation and growth in many tissues such as the liver, mammary glands and adrenals, embryogenesis and tissue differentiation. These effects largely result from the stimulation of DNA replication, gene transcriptions (including that for growth hormone), protein synthesis, and modulation of enzymes activities through their phosphorylation-dephosphorylation.

Regulation :

Post-absorptive blood glucose level does not directly stimulate insulin secretion. But its feedback probably increases the sensitivity of β cells to non-glucose factors like blood amino acids, growth hormone, cortisol, thyroxine and autonomic neurotransmitters. This may maintain the basal insulin secretion. The normal postprandial blood glucose level of 80-100 mg dl⁻¹ is the threshold stimulus for insulin release.

During intestinal absorption or infusion of glucose, both the rate of rise and the static high level of blood glucose stimulate β cells directly for the synthesis and release of insulin. In the *first phase* of response, the release of insulin rises sharply within a minute of hyperglycemia, reaches its peak in a couple of minutes, but declines in about 10 minutes.

This phase probably results from a direct binding of glucose to glucoreceptors on the β cell membrane, thereby stimulating the release of stored insulin from probably a small intracellular pool of the hormone. The *second phase* follows with a less steep but more sustained rise in insulin release from mainly a larger intracellular pool. This is probably brought about by a glucose metabolite which stimulates both the synthesis and the release of insulin and also potentiates the action of non-glucose stimuli on insulin secretion. A blood glucose level of 300 mg or more is required for attaining the V_{max} of insulin release.

Glucose probably depolarizes the plasma membrane by lowering its permeability to K^+ and consequently increases Ca^{2+} influx into β cells. Ca^{2+} then stimulates the microtubule-microfilament system and enhances exocytosis.

Insulin secretion is stimulated by a rise in blood amino acids, particularly arginine and leucine, but mostly in presence of glucose.

GIP stimulates insulin secretion in presence of hyperglycemia, but not at normal blood glucose levels (page 589). β -Adrenergic agonists and GIP increase intracellular cAMP which enhances Ca^{2+} translocation from organelles to cytosol and potentiates the β -cell response to glucose. Adrenergic sympathetic neurons and adrenaline inhibit the glucose-induced release of insulin by their α -adrenergic effect. Cholinergic vagal efferents enhance the insulin release.

Comparative aspects

Dogs and cats are very insulin-sensitive and depend more on insulin than on glucagon. Cattle, sheep and goat are less dependent on insulin. In reptiles and birds, α cells predominate over β cells. They secrete more glucagon than insulin, show high resistance to insulin and turn temporarily hypoglycemic on pancreatectomy. Urodele amphibians like salamanders secrete only insulin from pure β

cell islets; anuran amphibians like frogs possess mixed islets secreting some glucagon and are consequently less insulin-sensitive than urodeles.

18.18 DIABETES MELLITUS

Diabetes mellitus results from a failure of either the secretion or the action of insulin. An additional factor consists of a hypersecretion of glucagon.

Diabetes mellitus is characterized by a decline in glucose utilization, poor glucose tolerance, hyperglycemia, glucosuria, cataract, enhanced fat and protein catabolism, low RQ, ketosis, hypercholesterolemia, atherosclerosis, thickening of vascular membranes, peripheral neuropathy, autonomic and sensory-motor dysfunctions, polyuria, polydipsia and in extreme cases, coma and death.

Insulin-dependent diabetes mellitus (IDDM)

This is also known as the *type I or juvenile-onset diabetes*. It results from a failure of β cells to secrete insulin and a consequent fall in the blood insulin/glucagon ratio. Usually it starts in the young abruptly in a severe form. The patient is usually thin and underweight, shows all the symptoms described above, is prone to ketosis, responds well to insulin therapy, and is very susceptible to hypoglycemia in case of insulin overdosage. The plasma insulin level is abnormally low and fails to rise as expected in response to hyperglycemia. Predisposing factors are the diabetes genes, associated with the HLA genes and probably linked to autoimmune reactions against islet tissue and to increased β -cell vulnerability to mumps, coxsackie B-4 and other viruses.

Non-insulin-dependent diabetes mellitus (NIDDM)

Also known as the *type II or adult-onset diabetes mellitus*, it arises from a peripheral

insensitivity to insulin. It is relatively mild and starts slowly at an advanced age. The fault lies in a reduced binding of insulin to its receptor; the latter has a poor concentration on the target cell membranes due to down-regulation. The patient is usually obese and overweight, not prone to ketosis, not responsive to insulin therapy, and not easily susceptible to insulin-overdose hypoglycemia. Atherosclerosis, vascular thickenings, neuronal dysfunctions and retinal degeneration are common. The plasma insulin level is either normal or high, and overshoots its normal peak after glucose administration. On reducing the obesity, the concentration of insulin receptors rises on target cells, tissue responsiveness to insulin is enhanced, glucose tolerance is improved and the plasma insulin level falls. The disease may involve a genetic factor not associated with HLA genes or autoimmune responses.

Metabolic changes in diabetes

1. *Hyperglycemia* results from (a) reduced glucose uptake by tissues like muscles and adipose tissues owing to the decreased membrane transport of glucose, (b) reduced glycolysis due to the failure of insulin-induced synthesis of phosphofructokinase and pyru-

vate kinase of that pathway, (c) increased gluconeogenesis because of the decline in the insulin-mediated repression of pyruvate carboxylase and PEP carboxykinase and in the insulin-mediated inactivation of fructose 1,6-bisphosphatase (page 346), and (d) increased gluconeogenesis due to an activation of pyruvate carboxylase by acetyl-CoA from enhanced β -oxidation (Fig. 18.30).

2. *Glucosuria* results from the rise of blood sugar above its renal threshold. The urinary glucose osmotically increases the urine volume (*polyuria*). The consequent water loss enhances thirst (*polydipsia*).

3. *Glycosylated hemoglobins* (page 354), particularly HbA_{1c}, rise 2-3 fold in the blood in prolonged and uncontrolled diabetes due to a nonenzymatic glycosylation of the β chains of hemoglobin with glucose, glucose 6-phosphate or fructose 1,6-bisphosphate. The glycosylated β chain consequently carries respectively a deoxyfructose, deoxyfructose 6-phosphate or deoxyfructose 1,6-bisphosphate residue, linked by an N-glycosidic bond to the α -NH₂ group of the N-terminal valine. Such glycohemoglobins differ from normal hemoglobin in O₂ affinity and may consequently cause tissue hypoxia, contributing probably to

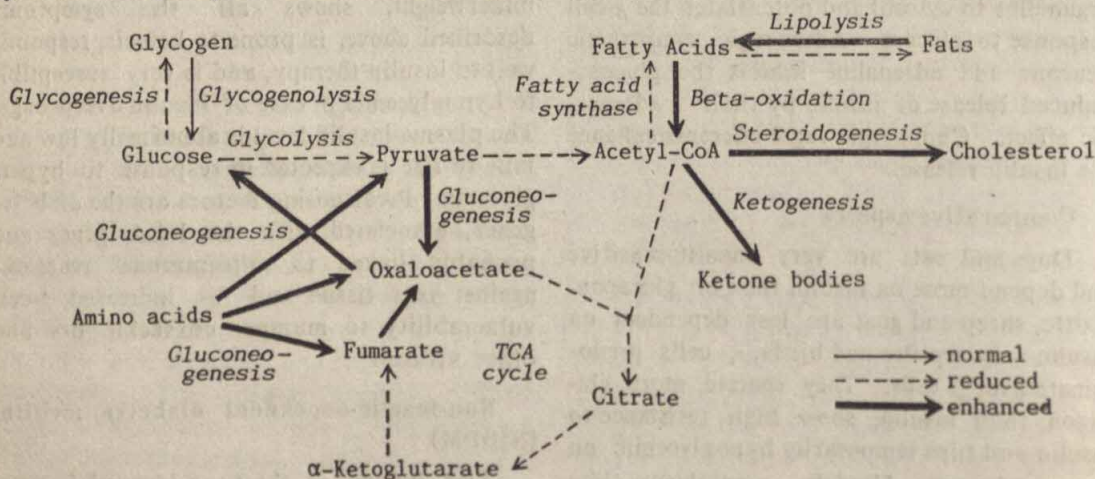


Fig. 18.30. Metabolic changes in diabetes mellitus.

some of the renal, vascular, neuronal and ophthalmic symptoms in diabetes. Estimation of blood glycohemoglobin finds clinical application in assessing diabetes because of the positive association of the blood HbA_{1c} concentration with the degree of hyperglycemia, its significant linear correlation with the peak of the glucose tolerance curve, and the prolonged persistence of glycohemoglobins in the blood.

4. *Nonenzymatic glycosylations* of lens α -crystallin, serum albumin and collagens are also increased. These may lead to chronic complications like cataract, glomerular basement membrane thickening, and thickening and morphological alterations of vascular membranes.

5. *Sorbitol pathway* (pages 347-348) is accelerated in diabetes, increasing the conversion of glucose to fructose and sorbitol, and causing their accumulation in tissues like the lens and nerve tissue. This results from the high intracellular glucose in those tissues and a high $\text{NADPH}/\text{NADP}^+$ ratio owing to the reduced utilization of NADPH in fatty acid synthesis—both glucose and NADPH are used in the sorbitol pathway. Sorbitol accumulation increases the osmotic influx of water in the lens and consequently damages its fibre structure, contributing to cataract. Peripheral neuropathy and other neuronal symptoms may result partly from changes in myoinositol due to sorbitol accumulation.

6. *Lipemia* (high plasma FFA) follows an enhanced lipolysis in adipose tissues due to the fall in the insulin-mediated inhibition of the hormone-sensitive lipase. This may lead to *fatty liver*.

7. *Ketosis* results from an enhanced production and a reduced removal of acetyl-CoA, because (a) an uninhibited activity of the adipose tissue lipase increases the availability of fatty acids for β -oxidation into acetyl-CoA, (b) increased gluconeogenesis lowers the mitochondrial concentration of oxaloacetate

needed for the TCA cycle and consequently decreases the ability to oxidize acetyl-CoA through that cycle, (c) acetyl-CoA is less utilized in fatty acid synthesis because of the decline in the synthesis and activity of acetyl-CoA carboxylase and NADPH-generating dehydrogenases in insulin deficiency, and the feedback inhibition of acetyl-CoA carboxylase by high levels of fatty acids, and (d) the acetyl-CoA, consequently accumulated in mitochondria, increases the activity of HMG-CoA synthase for the rate-limiting step of ketogenesis.

8. *Diabetic coma* may result from the depression of brain centres by acidosis due to excessive ketosis.

9. *Hypercholesterolemia* and *atherosclerosis* result from excessive biosynthesis of cholesterol from acetyl-CoA.

10. *Protein synthesis* is reduced while urea synthesis, urinary elimination of nitrogen and wasting of tissue proteins are enhanced.

18.19 THYROID HORMONES

Thyroid hormones, viz., *thyroxine* (T_4 or 3,5,3',5'-tetraiodothyronine) and 3,5,3'-triiodothyronine (T_3), are iodinated amino acids (Fig. 18.31). They are secreted by the follicular cells; parafollicular C cells of the thyroid secrete *calcitonin* (page 608-609).

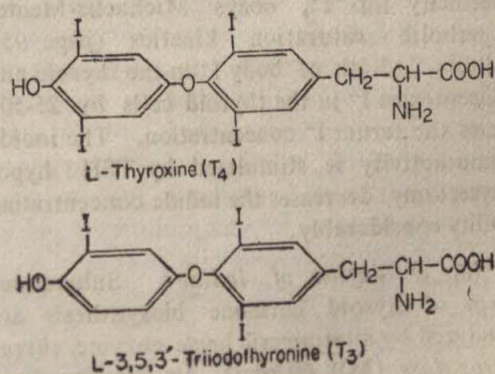


Fig. 18.31. Thyroid hormones.

Synthesis

(a) *Synthesis of thyroglobulin*: Thyroid hormones are synthesized by the iodination of tyrosine residues of a large protein called thyroglobulin. The latter is a 19S dimeric glycoprotein (MW 660 kdal) having 115 tyrosine residues and more than 20 oligosaccharide chains. Thyroglobulin is translated by polysomes on the granular endoplasmic reticulum of thyroid cells. The glycosylation of thyroglobulin is started in the smooth endoplasmic reticulum with the incorporation of mannose and is completed in the Golgi cisternae where other sugars such as fucose, sialic acid and galactose are incorporated into the oligosaccharide sidechains. Small, electron-lucent, membrane-bound vesicles, containing thyroglobulin, are then pinched off from the Golgi cisternae. These vesicles marginate towards the apical plasma membrane and fuse with it, releasing their contents into the colloid of the thyroid follicle.

(b) *Collection of iodide*: Thyroid follicular cells actively collect inorganic iodides (I^-) from the blood against steep electrochemical gradients with the help of an *iodide pump*. This iodide transporter is located in the basal plasma membrane in association with Na^+ - K^+ -dependent ATPase and requires a simultaneous activity of the Na^+ -pump. The iodide pump requires ATP, shows substrate-specificity for I^- , obeys Michaelis-Menten hyperbolic saturation kinetics (page 95), collects $\frac{1}{3}$ rd- $\frac{1}{4}$ th of body I^- in the thyroid and concentrates I^- in the thyroid cells by 25-500 times the serum I^- concentration. The iodide pump activity is stimulated by TSH; hypophysectomy decreases the iodide concentrating ability considerably.

(c) *Oxidation of iodide*: Subsequent steps of thyroid hormone biosynthesis are catalyzed by a tetrameric heme enzyme, *thyroperoxidase* (MW 60 kdal). Its inactive form is synthesized on the ER and packaged by the

Golgi cisternae into vesicles pinched off from them. It is activated during the fusion of the vesicle with the apical plasma membrane. Thyroperoxidase requires an NADPH-dependent H_2O_2 -generating enzyme system for the supply of H_2O_2 for its activity. At the colloid-membrane interface, thyroperoxidase binds I^- and thyroglobulin at distinct sites of its molecule. It then utilizes H_2O_2 to oxidize the enzyme-bound I^- to "active iodine" which may be hypoiodite (IO^-), iodinium (I^+) or free iodine radical ($I\cdot$).

(d) *Iodination of tyrosine*: Thyroperoxidase next transfers the "active iodine" from its iodide-binding site to a tyrosine residue of the enzyme-bound thyroglobulin to substitute a hydrogen attached to the phenyl ring.

Thyroperoxidase probably uses H_2O_2 to oxidize the tyrosine residue to a free tyrosine radical before its interaction with "active iodine". Successive iodinations at C^3 and C^5 of tyrosine produce monoiodotyrosine (MIT) and diiodotyrosine (DIT) (Fig. 18.32). About $\frac{1}{4}$ th of the tyrosine residues of thyroglobulin may thus change into iodotyrosine residues. Thyroperoxidase may also iodinate a few histidine residues to N^3 -monoiodohistidine (MIH); it can also iodinate some other proteins like serum albumin. Some other peroxidases such as lactoperoxidase can also similarly iodinate thyroglobulin.

(e) *Coupling of iodotyrosines*: A T_4 or a T_3 residue is next produced in thyroglobulin by the oxidative coupling of respectively two DIT residues or one DIT and one MIT. Thyroperoxidase probably oxidizes iodotyrosine residues with H_2O_2 to produce free iodotyrosine radicals. Probably the C—C bond between the phenyl ring of an iodotyrosyl radical and its alanine sidechain is next cleaved and this phenyl ring is transferred to the phenolic OH of another diiodotyrosyl radical, occurring in the same or another peptide chain of the thyroglobulin

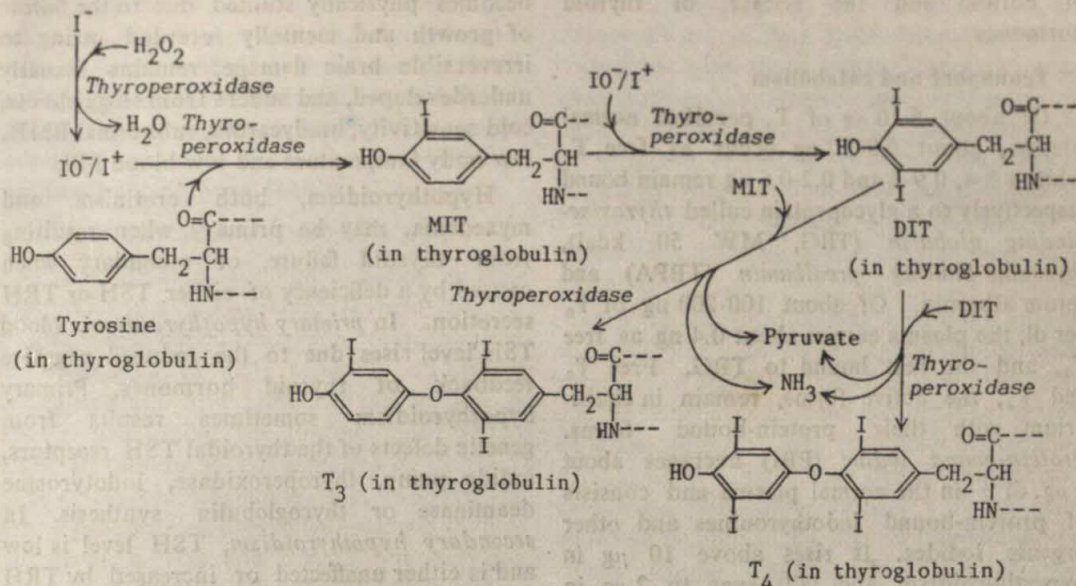


Fig. 18.32. Biosynthesis of thyroid hormones.

molecule, to produce an iodothyronine residue. The peptide chain of thyroglobulin may probably be broken during the transfer of the aromatic ring of its iodotyrosine residue to that of the other chain, because noniodinated preparations of thyroglobulin contain very little of small peptides while iodinated thyroglobulin preparations are rich in them. Anyway, the coupling reaction leaves an alanine, dehydroalanine or serine as a remnant of the cleaved iodotyrosine, either in the peptide chain or freed from the latter; this remnant is catabolized to pyruvate and ammonia. Such coupling produces on average only about 2-5 T_4 residues and 1-2 T_3 residues in a thyroglobulin molecule.

TSH stimulates the synthesis of thyroglobulin and all the reactions for forming iodothyronines.

Storage and release

Thyroid hormones are stored in the thyroglobulin molecules of the colloid of thyroid follicles. Organic iodine constitutes about

95% of the total of about 6 mg thyroidal iodine content. Normally, T_4 , T_3 , MIT and DIT contain respectively 35, 7, 25 and 40 percent of the thyroidal organic iodine. Small amounts of iodine occur in biologically inactive reverse T_3 (3,3',5'- T_3) and 3,3'-diiodothyronine.

Thyroid cells pinocytose colloid droplets which coalesce with lysosomes. Lysosomal acid proteases hydrolyze thyroglobulin to release iodothyronines and iodotyrosines. Glutathione-thyroglobulin transhydrogenase may cleave reductively some S-S linkages of thyroglobulin. Iodothyronines pass into the blood probably by facilitated diffusion across the basal cell membrane—about 50-70 μg of thyroid hormone iodide are normally secreted daily. Microsomal deiodinases use NADPH to deiodinate iodotyrosines. The released iodine is recycled into tyrosine iodination. TSH increases the number of microvilli and the activity of microtubules in the apical part of the thyroid cell to enhance the pinocytosis

of colloid and the release of thyroid hormones.

Transport and catabolism

Of about 5-10 μg of T_4 per dl of normal plasma, about 0.8-2.2 ng occur as free T_4 , while 1.8-4, 0.9-2 and 0.2-0.6 μg remain bound respectively to a glycoprotein called *thyroxine-binding globulin* (TBG, MW 50 kdal), *thyroxine-binding prealbumin* (TBPA) and serum albumin. Of about 100-200 ng of T_3 per dl, the plasma carries about 0.4 ng as free T_3 , and the rest bound to TBG. Free T_3 and T_4 , the active forms, remain in equilibrium with their protein-bound forms. *Protein-bound iodine* (PBI) averages about 8 $\mu\text{g dl}^{-1}$ in the normal plasma and consists of protein-bound iodothyronines and other organic iodides. It rises above 10 μg in hyperthyroidism and falls even to 2 μg in hypothyroidism.

Liver, kidneys, muscles and heart deaminate iodothyronines into iodothyropyruvates and oxidatively decarboxylate them to iodothyroacetates. Iodothyronines and their products are deiodinated by tissue deiodinases, conjugated with glucuronate, sulfate or methyl groups, and excreted in the bile and urine.

About 25% of the circulating T_3 comes from thyroid; the rest is formed by partial deiodination of T_4 in peripheral tissue.

Clinical conditions

Myxedema :

A deficiency of thyroid hormones (hypothyroidism) produces myxedema in adults. It is characterized by low BMR, low blood PBI, puffy appearance, bradycardia, low body temperature, physical and mental sluggishness, weight gain, somnolence and hypercholesterolemia. The puffy appearance is due to the deposition of proteoglycans in the skin.

Cretinism :

Hypothyroidism produces cretinism in infants and children. The afflicted child

becomes physically stunted due to the failure of growth and mentally retarded owing to irreversible brain damage, remains sexually underdeveloped, and suffers from sluggishness, cold sensitivity, bradycardia, subnormal BMR, low body temperature and low blood PBI.

Hypothyroidism, both cretinism and myxedema, may be primary when resulting from thyroid failure, or secondary when caused by a deficiency of either TSH or TRH secretion. In *primary hypothyroidism*, blood TSH level rises due to the reduced negative feedback of thyroid hormones. Primary hypothyroidism sometimes results from genetic defects of the thyroidal TSH receptors, iodide pump, thyroperoxidase, iodothyrosine deaminase or thyroglobulin synthesis. In *secondary hypothyroidism*, TSH level is low and is either unaffected or increased by TRH infusion according as the defect lies in the pituitary or the hypothalamus respectively.

Simple goitre :

Dietary deficiency of iodide decreases the synthesis and secretion of thyroid hormones, lowers the blood T_4 level and decreases the negative feedback effect of T_4 on TSH secretion. TSH secretion consequently rises and enlarges the thyroid (goitre). Symptoms of hypothyroidism (myxedema or cretinism) also appear. Such *iodine deficiency goitres* may affect large sections of both human and bovine populations (*endemic goitre*) in mountain regions with poor soil iodides.

Simple goitre may also result from the excessive intake of foods rich in antithyroid substances which block the thyroidal iodide uptake or hormone synthesis; e.g., edible seaweeds rich in iodides; mustard leaves and cabbage rich in thiocyanates; mustard seeds, cabbage, kohlrabi and turnip rich in thioglycosides.

Hashimoto's lymphocytic thyroiditis :

In this disease, autoimmunization against thyroidal antigens leads to the destruction

of thyroid tissue. Antithyroid immunoglobulins reach a high serum titre, lymphocytes infiltrate the thyroid, iodothyronine synthesis declines, TSH is consequently oversecreted and goitre results. Hyperthyroidism develops in the mid-course of the disease, but is finally replaced by hypothyroidism.

Grave's disease or exophthalmic goitre :

In this disease, autoimmunization against thyroid components produces *thyroid-stimulating IgG*, also called *long-acting thyroid stimulator*. It binds to TSH receptors on thyroid cell membrane and simulates the action of TSH in stimulating the thyroid. But unlike TSH, its action is not inhibited by the feedback of thyroid hormones. So, its prolonged action produces an overactive enlarged thyroid (*thyrotoxic goitre*) and thyroid oversecretion (*hyperthyroidism*). Hyperthyroidism increases the blood PBI, Q_{O_2} of tissues, BMR, body temperature, sweating, heat sensitivity, heart rate, blood pressure, pulse pressure and blood sugar; it also produces glucosuria, negative N balance, weight loss, tremor, restlessness, inability to sleep and psychosis. A protrusion of eyeballs (*exophthalmos*) results from mucoprotein deposition and edema in the retrobulbar tissue due probably to an exophthalmos-producing IgG.

Actions

Thyroid hormones are transported into their target cells by a carrier-mediated active transport system of the cell membrane. The target cells include hepatocytes, renal cells, cardiac cells, neurons, lymphocytes and pituitary somatotrophs. Inside the cells, T_4 is mostly changed by deiodinase to T_3 which has a shorter half-life and about 3-4 fold higher biological potency.

(a) T_4 and T_3 pass into the nucleus and bind directly to specific high-affinity *nuclear receptors* which are nonhistone chromatin proteins (MW $\approx$ 50 kdal) of specific genes and have far higher affinities for T_3 than for T_4 .

This receptor-hormone binding augments the actions of the nuclear DNA-dependent RNA polymerases on those genes, increases gene transcription, enhances the synthesis of hnRNA and mRNA, and consequently induces the synthesis of specific proteins (e.g., hypophysial growth hormone and hepatic glucokinase, glycerol kinase, arginase, Na^+K^+ -dependent ATPase, malic enzyme and α_{2u} -globulin).

(b) In addition, thyroid hormones appear to bind directly to some high-affinity protein receptors of the *inner mitochondrial membrane* and may thereby affect the synthesis or action of some mitochondrial enzymes. Mitochondrial glycerol 3-phosphate dehydrogenase and carbamoyl phosphate synthase I have been shown to be induced by thyroid hormones.

(c) Besides affecting gene transcription, thyroid hormones may probably stimulate *translation of proteins* directly by augmenting the binding of amino acyl-tRNA complex to the polysome, the activity of peptidyl transferase or that of the translocase.

(d) Thyroid hormones, particularly T_3 , seem also to bind to specific receptors on the plasma membrane. This may probably enhance transmembrane transport of some substrates like 2-deoxyglucose and some amino acids by activating their membrane transporters.

(e) Thyroid hormones potentiate some of the β -adrenergic effects of catecholamines (e.g., adipose tissue lipolysis) by inducing and thereby *up-regulating the β -adrenergic receptors* on some tissue cells and also by enhancing the coupling of these receptors with the GTP-dependent regulatory complex which acts as the transducer for activating adenylate cyclase (page 578).

The biological effects of thyroid hormones are summarized below :

1. *Calorigenic effect* : Thyroid hormones considerably enhance O_2 consumption and

oxygen coefficient (Q_{O_2}) in many tissues except brain, testes and spleen, and increase the heat production and BMR of the organism. This results partly from the induction of glycerol 3-phosphate dehydrogenase and other enzymes involved in mitochondrial oxidations, but probably more from the induction of the membrane Na^+K^+ -ATPase which hydrolyzes ATP for the transmembrane extrusion of Na^+ , leading to enhanced heat production, O_2 consumption and oxidative phosphorylation. The earlier concept of the thyroxine-mediated uncoupling of mitochondrial oxidation and phosphorylation seems improbable *in vivo*.

2. *Growth and differentiation effects*: Thyroid hormones promote N retention, protein synthesis, positive N balance, body growth and tissue differentiation, by enhancing the transcription of genes for tissue proteins and growth hormone. The effects on growth and differentiation are accompanied or preceded by the induction of synthesis of many proteins including enzymes. T_4 administration to pregnant mammals either induces the synthesis or increases the activities of glucokinase, glucose 6-phosphatase, glycerol 3-phosphate dehydrogenase, cytochrome oxidase, arginase, malic enzyme, pyruvate carboxylase, PEP carboxykinase and glycerokinase in the liver of their offsprings. Hypothyroidism at an early age produces cretinism with growth failure, congenital defects and severe mental retardation.

Similar actions of thyroid hormones stimulate metamorphosis in Amphibia like tadpoles. T_4 administration causes their premature metamorphosis while thiouracil treatment produces a failure of metamorphosis. In metamorphosing tadpoles, thyroid hormones induce many enzymes such as hyaluronidase and lysosomal hydrolases for the regression of tail and gills, and urea cycle enzymes (particularly carbamoyl phosphate synthase I and

arginase) for the change from ammonotelism to ureotelism.

3. *Effects on carbohydrate metabolism*: Thyroid hormones raise the blood sugar, reduce glucose tolerance and increase glucose utilization. Hyperthyroidism produces hyperglycemia and glucosuria. Thyroidectomy reduces the severity of pancreatic diabetes while T_4 administration after subtotal pancreatectomy produces diabetes in experimental animals. Thyroid hormones, therefore, are antagonistic to insulin. (a) Thyroid hormones enhance the intestinal absorption rate of glucose. (b) They increase hepatic glycogenolysis, because they enhance the activity of glucose 6-phosphatase and also potentiate the glycogenolytic effect of adrenaline by up-regulating the β -adrenergic receptors on the hepatic cell membrane. (c) Thyroid hormones increase hepatic gluconeogenesis by enhancing the activities of pyruvate carboxylase and PEPCK carboxykinase. (d) They accelerate the operation of the pentose phosphate pathway by increasing the activity of glucose 6-phosphate dehydrogenase. (e) They enhance glucose oxidation by enhancing the activities of succinoxidase and other mitochondrial oxidoreductases.

4. *Effects on lipid metabolism*: (a) Thyroid hormones potentiate the lipolytic action of adrenaline by up regulating the β -adrenergic receptors on the adipocyte cell membrane. Thus, they indirectly enhance adipose tissue lipolysis and the blood level of free fatty acids. (b) They may stimulate lipogenesis by enhancing the activities of malic enzyme, ATP-citrate lyase and glucose 6-phosphate dehydrogenase. (c) Thyroid hormones lower serum cholesterol by increasing its conversion to bile acids and elimination in bile. Hypothyroidism and hyperthyroidism are respectively accompanied by hypercholesterolemia and hypocholesterolemia.

5. *Effects on protein metabolism*: Thyroid hormones produce protein anabolic effects in

moderate doses, but inhibit protein synthesis and increase blood amino acid level and urinary NPN in large doses. Hyperthyroidism causes negative N balance. In different animals, thyroid hormones induce L-glutamate dehydrogenase and urea cycle enzymes in the liver.

Antithyroid substances

These substances inhibit the synthesis, release or action of thyroid hormones. Those inhibiting the thyroid secretion may act as *goitrogens* because the fall in blood T_4 produced by them enhances TSH secretion leading to the enlargement of the thyroid. *Nongoitrogenic antithyroid substances* produce hypothyroidism by inhibiting the peripheral action of thyroid hormones, but do not produce goitre. Antithyroid substances are classified according to their modes of action.

1. Inhibitors of iodide uptake :

When present in high concentrations, some monovalent anions having almost the same van der Waals radii as iodide, compete with iodide to occupy its binding site on the iodide pump, thereby increase the K_m of the latter for iodide and consequently decrease the active iodide collection by the thyroid to lower the thyroid:serum free iodide ratio (*T/S ratio*) to near-unity; this reduces the synthesis of thyroid hormones. Such anions include pertechnetate (TcO_4^-), perchlorate (ClO_4^-),

selenocyanate ($SeCN^-$), thiocyanate (SCN^-), tetrafluoroborate (BF_4^-), nitrite (NO_2^-) and bromide (Br^-), in the descending order of their inhibitory actions on the iodide pump. Except thiocyanate and selenocyanate, others are concentrated into the thyroid cell by the iodide pump. Cabbage and mustard leaves contain thiocyanate; mustard oil, cabbage leaves, turnip and kohlrabi contain isothiocyanate which can be metabolized to thiocyanate; a cyanogenic glycoside of cassava may be changed to thiocyanate by colonic bacteria. So, regular excess intake of such foods may produce hypothyroidism and goitre. Iodide itself, though antithyroid at high concentrations, does not inhibit the iodide pump; an enhanced iodide intake or a high serum iodide concentration overcomes the goitrogenic effects of iodide pump inhibitors.

The cardiac glycoside ouabain inhibits the thyroidal iodide uptake by inhibiting Na^+K^+ -ATPase required for running the iodide pump. Phenylbutazone can also reduce the iodide uptake by thyroid. Both these may produce hypothyroidism and goitre in humans.

In case of radioactive iodide ingestion or contamination, perchlorate is administered in large doses to the patients to inhibit the collection and consequent damaging action of radioiodides in the thyroid cell. Perchlorate is used in studying the kinetics of iodothyro-

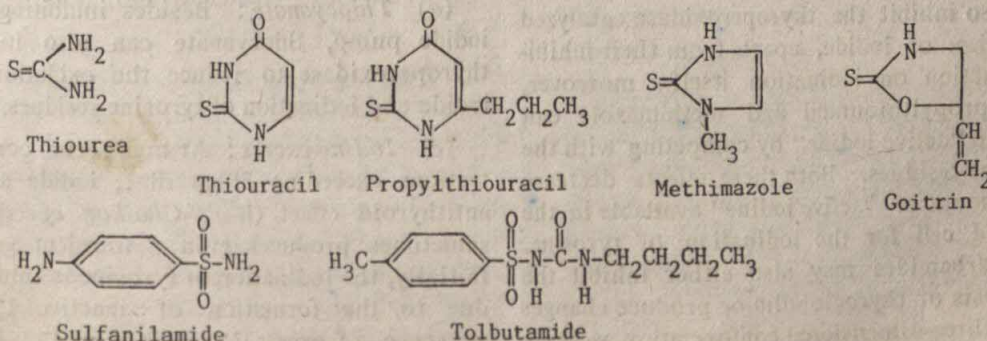


Fig. 18 33. Some antithyroid substances.

nine secretion. Radioisotopic pertechnetate may be administered to get an image of the thyroid with a scintillation scanner or a gamma camera. In the *perchlorate-discharge test* for the ability to synthesize iodothyronine, a radioiodide of short half-life (e.g., $^{131}\text{I}^-$) is allowed to be concentrated in the thyroid following its administration, further radioiodide uptake is then blocked by administering perchlorate and the rate of radioiodine release from the thyroid is measured for an interval. Perchlorate and thiocyanate, though effective in reducing hyperthyroidism, are seldom used in its treatment because of their toxicity on long-term use and ineffectiveness in case of a rise in serum iodide.

2. Inhibitors of iodination and coupling :

(a) *Thiocarbamides* : Compounds possessing a thiocarbamide group ($\text{N}-\text{C}-\text{SH}$) may inhibit thyroperoxidase-catalyzed reactions to produce hypothyroidism and goitre. They include thiourea, thiouracil, propylthiouracil, methimazole and carbimazole (Fig. 18.33). Cabbage, mustard seeds, turnip and kohlrabi roots contain thioglycosides called *progoitrins* which are hydrolyzed by glycosidases of colonic bacteria into active, thiocarbamide-bearing antithyroid substances called *goitrins* (e.g., L-5-vinyl-2-thio-oxazolidone from turnip progoitrin). Thiocarbamides inhibit in an ascending order the iodination of tyrosine to MIT, the iodination of MIT to DIT, and the coupling of iodothyronines. Thiocarbamides can also inhibit the thyroperoxidase-catalyzed oxidation of iodide, apart from their inhibitory action on iodination itself; moreover, both propylthiouracil and methimazole can bind up "active iodine" by competing with the tyrosine residues. Both these effects decrease the amount of "active iodine" available in the thyroid cell for the iodination of tyrosine. Thiocarbamides may also either inhibit the synthesis of thyroglobulin or produce changes in its three-dimensional conformation weakening its dine-binding capacity. High doses of

iodide decrease the antithyroid effects of thiocarbamides only slightly.

Propylthiouracil, methimazole and carbimazole are used for reducing the synthesis and secretion of thyroid hormones in hyperthyroid patients. They are much less toxic than the iodide pump inhibitors. They are very useful in Grave's disease caused by thyroid-stimulating immunoglobulins (TSI), because they can reduce the autoimmune formation of TSI by their immunosuppressive actions. Propylthiouracil is further effective because it inhibits the peripheral deiodination of T_4 to more active T_3 . But thiouracil treatment of pregnant mothers may cause goitre in the newborn infant.

(b) *Aminoheterocyclic compounds* : Tolbutamide, sulfanilamide, carbutamide, sulfonilurea, para-aminobenzoate and para-aminosalicylate may inhibit thyroperoxidase to reduce the iodination of tyrosine and coupling of iodothyronines, but are far weaker than thiocarbamides. Unlike the latter, some aminoheterocyclic compounds have their antithyroid effects potentiated by dietary iodide supplements, probably because they have a different mode of action on thyroperoxidase.

(c) *Substituted phenols* : Compounds like resorcinol and phloroglucinol inhibit thyroperoxidase. Resorcinol is a more potent goitrogen than methimazole and other thiocarbamides.

(d) *Thiocyanate* : Besides inhibiting the iodide pump, thiocyanate can also inhibit thyroperoxidase to reduce the oxidation of iodide and iodination of tyrosine residues.

(e) *Iodine excess* : At high serum concentrations exceeding $30 \mu\text{g dl}^{-1}$, iodide exerts antithyroid effect (*Wolff-Chaikoff effect*) and sometimes produces even a transient goitre. Initially, the iodination of tyrosine is inhibited due to the formation of inactive I_3^- in presence of excess I^- : $\text{I}^- + \text{I}_2 \rightleftharpoons \text{I}_3^-$. Long-term effects consist of a decrease in the

TSH-induced rise in cAMP in thyroid cells with a consequent inhibition of TSH action, inhibition of pinocytosis of colloid droplets into the thyroid cell, reduction of proteolysis of the engulfed thyroglobulin and the consequent decline in the release of thyroid hormones. Iodide is frequently administered to hyperthyroid patients for a short interval before subtotal thyroidectomy so as to bring down the blood thyroid hormone level, reduce the chance of acute postoperative hyperthyroidism, enhance the firmness of thyroid and decrease its vascularity.

3. Inhibitors of hormone release :

Pinocytosis of colloid droplets through the apical cell membrane requires the actions of microtubules and microfilaments. Colchicine, vincristine and vinblastine inhibit the polymerization of tubulin into microtubules while cytochalasin inhibits microfilament activities. Lithium salts, on the other hand, inhibit the transport of thyroid hormones across the basal membrane of thyroid cells. All these substances reduce the release of hormones from the thyroid.

4. Inhibitors of T_4 - T_3 conversion :

Propanolol, propylthiouracil, amiodarone and oral cholecystographic agents like iopanoic acid inhibit the 5'-monodeiodinase-catalyzed conversion of T_4 to more active T_3 in peripheral tissues. They thus decrease the serum T_3 concentration and the peripheral actions of the hormones.

Regulation

Thyrotropin of anterior pituitary stimulates the growth of the thyroid tissue, collection of iodide by the thyroid, iodination of tyrosine residues of thyroglobulin, coupling of iodotyrosines and release of thyroid hormones by the thyroid. The secretion of thyrotropin is in turn largely stimulated by hypothalamic TRH and inhibited by the negative feedback of high blood levels of thyroid hormones (pages 598-599).

18.20. REGULATION OF BLOOD SUGAR

The carbohydrate metabolism is regulated and the normal blood sugar is maintained by a balance between the actions of insulin and those of glucocorticoids, growth hormone, adrenaline, glucagon and thyroid hormones.

1. Insulin

Insulin is of prime importance in regulating the blood sugar. A pancreatic β cell tumor or an insulin overdose produces *hyperinsulinism* with severe hypoglycemia, consequent depression of brain centres and even a loss of consciousness (*insulin shock*). Pancreatectomy, injection of alloxan or pituitary extracts, or a clinical failure of insulin secretion causes *diabetes mellitus* with hyperglycemia, glucosuria, poor glucose tolerance, ketosis and even coma and death.

A rise in blood sugar level stimulates insulin secretion. Insulin lowers the blood sugar in several ways (pages 632-633 for details).

(a) It increases glucose uptake by muscles, adipocytes and other extrahepatic tissues.

(b) It enhances utilization of glucose by promoting glycolysis and aerobic metabolism of pyruvate in tissues and also by stimulating lipogenesis from glucose in adipocytes.

(c) It reduces both glycogenolysis and gluconeogenesis in the liver and thereby decreases the addition of glucose to the blood.

(d) It stimulates glycogenesis in the liver to enhance the storage of carbohydrates as liver glycogen.

2. Glucagon

Secretion of glucagon is stimulated by a fall in blood sugar level. Glucagon is antagonistic to insulin and increases the blood sugar, lowers the liver glycogen and may even produce glucosuria because of its following effects (pages 628-629 for details).

(a) Glucagon enhances glycogenolysis in the liver.

(b) It increases hepatic gluconeogenesis.

(c) It decreases hepatic glycogenesis and thus reduces the removal of blood glucose by the liver.

3. Adrenaline

Secreted from adrenal medulla during hypoglycemia, cold and exercise, adrenaline increases both blood sugar and blood lactate levels. Its actions on the carbohydrate metabolism may be summarized as follows (pages 613-614 for details).

- (a) It stimulates glycogenolysis in the liver.
- (b) Adrenaline stimulates hepatic gluconeogenesis also.
- (c) It increases the blood lactate by promoting muscle glycolysis.
- (d) It reduces the utilization of blood glucose by increasing adipose tissue lipolysis and consequently making more fatty acids available for energy production.

4. Glucocorticoids

Adrenal glucocorticoids like cortisol tend to raise the blood sugar. They help to maintain the liver glycogen during fasting. In *Cushing's syndrome*, excessive glucocorticoid secretion produces hyperglycemia, poor sugar tolerance and glucosuria; in *Addison's disease*, glucocorticoid deficiency causes hypoglycemia, insulin hypersensitivity and abnormally high sugar tolerance. Glucocorticoids act antagonistic to insulin and affect the carbohydrate metabolism and the blood sugar in the following ways (pages 617-618 for details).

- (a) Glucocorticoids increase gluconeogenesis in the liver from amino acids.
- (b) They decrease uptake and utilization of glucose by extrahepatic tissues like muscles.
- (c) They increase the hepatic glycogen storage by enhancing both gluconeogenesis and the action of glycogen synthase.

5. Thyroid hormones

In *Grave's disease*, hyperthyroidism leads to hyperglycemia, poor sugar tolerance and

glucosuria. Thyroidectomy reduces the severity of pancreatic diabetes in experimental animals. The thyroid hormones act against insulin and tend to raise the blood sugar and reduce sugar tolerance, mainly in the following ways (page 642 for details).

- (a) Thyroid hormones enhance the intestinal absorption of glucose.
- (b) They increase hepatic glycogenolysis.
- (c) They also stimulate gluconeogenesis in the liver.

6. Growth hormone

Growth hormone is antagonistic to insulin in most of its effects on the carbohydrate metabolism. In *gigantism* and *acromegaly*, excessive GH secretion causes hyperglycemia, glucosuria and poor sugar tolerance. In *Simmond's disease*, failure of GH secretion leads to hypoglycemia. Hypophysectomy reduces the severity of pancreatic diabetes in experimental animals while injections of anterior lobe extracts to subtotally pancreatectomized animals may produce permanent diabetes. *Houssay animals*, with both pituitary and pancreas removed, show poor sugar tolerance and severe insulin hypersensitivity. GH actions on the carbohydrate metabolism and the blood sugar are summarized below (page 605 for details).

- (a) GH has diabetogenic effects, because it raises the blood sugar by inhibiting the uptake and utilization of glucose by muscles and adipocytes, and increases hepatic gluconeogenesis.
- (b) It exerts an anti-insulin effect by reducing insulin-sensitivity.
- (c) It increases gluconeogenesis to enhance and maintain the liver glycogen level (glycostatic effect).

7. Prolactin

Prolactin has hyperglycemic, diabetogenic and anti-insulin effects like growth hormone.

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Corrigendum

The last three lines of column 2 on page 48 should be read as follows :

iodothyronines (the thyroid hormones T₄ and T₃), homocysteine (produced from methionine), ornithine and citrulline (urea cycle)

